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FORM PTO-1390 (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		P-208614.9 (PCT) (US)
		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/070484
INTERNATIONAL APPLICATION NO. PCT/US00/25408	INTERNATIONAL FILING DATE 15 SEP 00	PRIORITY DATE CLAIMED 17 SEP 99

TITLE OF INVENTION
INDOLE-CONTAINING and COMBRETASTATIN-RELATED ANTI-MITOTIC and TUBULIN POLYMERIZATION AGENTS

APPLICANT(S) FOR DO/EO/US
BAYLOR UNIVERSITY

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

(January 1999)

1007040484 - 101102

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U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/070484	PCT/US00/25408	P-208614.9 (PCT) (US)

	CALCULATIONS	PTO USE ONLY
17. [X] The following fees are submitted: **BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):** Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .... $970.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .... $840.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... $760.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) .... $670.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 970.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =	280	X $18.00	$ 5040.00	
Independent claims	- 3 =	40	X $78.00	$ 3120.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$ 260.00	
TOTAL OF ABOVE CALCULATIONS =				$ 8420.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 4210.00	
SUBTOTAL =				$	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 4210.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 4210.00	
				Amount to be: refunded	$
				charged	$

a. [X] A check in the amount of $ 4210.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. ______________ in the amount of $______________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 07-2400 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

SIGNATURE: Daniel S. Hodgins

NAME DANIEL S. HODGINS

31,026
REGISTRATION NUMBER

INDOLE-CONTAINING AND COMBRETASTATIN-RELATED ANTI-MITOTIC AND ANTI-TUBULIN POLYMERIZATION AGENTS

BACKGROUND OF THE INVENTION

Tubulin is currently among the most attractive therapeutic targets in new drug design for the treatment of solid tumors.[1c] The heralded success of vincristine and taxol along with the promise of combretastatin A-4 (CA-4) prodrug and dolastatin 10, to name just a few, have firmly established the clinical efficacy of these antimitotic agents for cancer treatment.

An aggressive chemotherapeutic strategy toward the treatment of solid-tumor cancers continues to rely on the development of architecturally new and biologically more potent anti-tumor, anti-mitotic agents which mediate their effect through a direct binding interaction with tubulin. A variety of clinically-promising compounds which demonstrate potent cytotoxicity and antitumor activity are known to effect their primary mode of action through an efficient inhibition of tubulin polymerization.[1] This class of compounds undergoes an initial interaction (binding) to the ubiquitous protein tubulin which in turn arrests the ability of tubulin to polymerize into microtubules which are essential components for cell maintenance and division.[2] During metaphase of the cell cycle, the nuclear membrane is broken down and the cytoskeletal protein tubulin is able to form centrosomes (also called microtubule organizing centers) and through polymerization and depolymerization of tubulin the dividing chromosomes are separated. Currently, the most recognized and clinically useful members of this class of antimitotic, antitumor agents are vinblastine and vincristine[3] along with taxol.[4] Additionally, the natural products rhizoxin,[5] combretastatin A-4 and A-2,[6] curacin A,[1] podophyllotoxin,[7] epothilones A and B,[8] dolastatin 10[9] and welwistatin[10] (to name just a few) as well as certain synthetic analogues including phenstatin,[11] the 2-styrylquinazolin-4(3H)-ones (SQO),[12] and highly oxygenated derivatives of *cis*- and *trans*-stilbene[13] and dihydrostilbene are all known to mediate their cytotoxic activity through a binding interaction with tubulin. The exact nature of this binding site interaction remains largely unknown, and definitely varies between the series of compounds. Photoaffinity labeling and other binding site elucidation techniques have identified several key binding sites on tubulin: colchicine site, vinca alkaloid site, and a site on the polymerized microtubule to which taxol binds.[1a,14]

SUMMARY OF THE INVENTION

An important basic and essential aspect of this work requires a detailed understanding, on the molecular level, of the "small molecule" binding domain of both the α and β subunits of tubulin. The tertiary structure of the α, β tubulin heterodimer was reported earlier this year by Downing and co-workers at a resolution of 3.7 Å using a technique known as electron crystallography.[15] This brilliant accomplishment culminates decades of work directed toward the elucidation of this structure and should facilitate the identification of small molecule binding sites, such as the colchicine site, through techniques such as photoaffinity and chemical affinity labeling.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates 3-(3', 4', 5' - trimethoxybenzoyl)-2-(4'-methoxyphenyl)-6-methoxybenzo[*b*]thiophene.

Figure 2 illustrates 2-(3', 4', 5'-trimethoxybenzoyl)-3-(4'-methoxyphenyl)-6-methoxybenzo[*b*]furan.

Figure 3 illustrates benzo[*b*]thiophene Phenol (BBT-OH).

Figure 4 illustrates benzo[*b*]thiophene prodrug (BBT-P).

Figure 5 illustrates *in vivo* biological data for benzo[*b*]thiophene prodrug (BBT-P).

Figure 6 illustrates a synthetic route for preparation of phenylindole derivatives.

Figure 7 illustrates a COSY NMR for 2-phenyl indole (aromatic region) a compound 31.

Figure 8 illustrates a cyclized isomer without aryl migration (no evidence for its formation).

Figure 9 illustrates a preparation of 2-phenylindole 31 in a one-pot reaction.

Figure 10 illustrates a designed synthetic route for preparation of indole-based analog.

Figure 11 illustrates a preparation of indole-based analog.

Figure 12 illustrates a synthesis of indole-based disodium prodrug salt.

Figure 13 illustrates another synthesis of indole-based disodium prodrug.

Figure 14 illustrates another synthesis of indole-based disodium prodrug.

Figure 15 illustrates a synthesis of indole based phosphoramidate prodrug.

Figure 16 illustrates another synthesis of indole-based disodium prodrug salt.

Figure 17A illustrates a combretastatin A-4 pro-drug.

Figure 17B illustrates a phosphoramidate analog 10.

Figure 18 illustrates a synthesis of phosphoramidate 10.

Figure 19 illustrates a model system used for phosphoramidate synthesis.

Figure 20 illustrates a synthesis of phosphoramidate 10 from from (Z)-3'-nitro combrestastatin analog 7B.

Figure 21 illustrates substituted 4-methoxyindole amines and/or phenols.

Figure 22 illustrates substituted 4-methoxyindole phosphate ester moieties and phosphoramidates.

Figure 23 illustrates further substituted 4-methoxyindole phosphate ester moieties and phosphoramidates.

Figure 24 illustrates substituted 6-methoxyindole amines and/or phenols.

Figure 25 illustrates substituted 6-methoxyindole phosphate ester moieties and phosphoramidates.

Figure 26 illustrates substituted 6-methoxyindole phosphate ester moieties and phosphoramidates.

Figure 27 illustrates substituted 4-methoxy-3-arylindole amines and/or phenols.

Figure 28 illustrates substituted 4-methoxy-3-arylindole phospate moieties and phosphoramidates.

Figure 29 illustrates further substituted 4-methoxy-3-arylindole phospate moieties and phosphoramidates.

Figure 30 illustrates 2-(4'-Methoxyphenyl)-3-(3", 4", 5"-trimethoxybenzoyl)-4-methoxyindole.

Figure 31 illustrates 2-(3', 4', 5'-Trimethoxybenzoyl)-3-(4"-methoxyphenyl)-6-methoxyindole.

Figure 32 illustrates 2-(3', 4', 5'-Trimethoxybenzoyl)-3-(4"-methoxyphenyl)-4-methoxyindole.

Figure 33 illustrates Disodium 2-(3'-phosphoramidate-4'-methoxyphenyl)-3-(3", 4", 5"-trimethoxybenzoyl)-6-methoxyindole.

Figure 34 illustrates 2-(3'-Hydroxy-4'-methoxyphenyl)-3-(3", 4", 5"-trimethoxybenzoyl)-4-methoxyindole.

Figure 35 illustrates 2-(3'-Amino-4'-methoxyphenyl)-3-(3", 4", 5"-trimethoxybenzoyl)-4-methoxyindole.

Figure 36 illustrates Disodium 2-[(4'-methoxyphenyl)-3'-O-phosphate]-3-(3", 4", 5"trimethoxybenzoyl)-4-methoxyindole.

Figure 37 illustrates 2-(3'-Diethylphosphoramidate-4'-methoxyphenyl)-3-(3", 4", 5"-trimethoxybenzoyl)-4-methoxyindole.

Figure 38 illustrates Disodium 2-(3'-phosphoramidate-4'-methoxyphenyl)-3-(3", 4", 5"-trimethoxybenzoyl)-4-methoxyindole.

Figure 39 illustrates 2-(3',4',5'-trimethoxybenzoyl)-3-(3"-hydroxy-4"-methoxyphenyl)-6-methoxyindole.

Figure 40 illustrates 2-(3',4',5'-trimethoxybenzoyl)-3-(3"-amino-4"-methoxyphenyl)-6-methoxyindole.

Figure 41 illustrates Disodium 2-(3',4',5'-trimethoxybenzoyl)-3-[(4"-methoxyphenyl-3"-*O*-phosphate)]-6-methoxyindole.

Figure 42 illustrates 2-(3',4',5'-trimethoxybenzoyl)-3-[(4"-methoxyphenyl-3"-diethylphosphoramidate)]-6-methoxyindole.

Figure 43 illustrates Disodium 2-(3',4',5'-trimethoxybenzoyl)-3-[(4"-methoxyphenyl-3"-phosphoramidate)]-6-methoxyindole.

Figure 44 illustrates 2-(3',4',5'-trimethoxybenzoyl)-3-(3"-hydroxy-4"-methoxyphenyl)-4-methoxyindole.

Figure 45 illustrates 2-(3',4',5'-trimethoxybenzoyl)-3-(3"-amino-4"-methoxyphenyl)-4-methoxyindole.

Figure 46 illustrates Disodium 2-(3',4',5'-trimethoxybenzoyl)-3-[(4"-methoxyphenyl-3"-*O*-phosphate)]-4-methoxyindole.

Figure 47 illustrates 2-(3',4',5'-trimethoxybenzoyl)-3-[(4"-methoxyphenyl-3"-diethylphosphoramidate)]-4-methoxyindole.

Figure 48 illustrates Disodium 2-(3',4',5'-trimethoxybenzoyl)-3-[(4"-methoxyphenyl-3"-phosphoramidate)]-4-methoxyindole.

Figure 49 illustrates substituted 3-phosphoramidate derivatives of combretastatin A-4.

Figure 50 illustrates Disodium (*Z*)-1-[(4'-methoxyphenyl)-3'-phosphoramidate]-2-(3",4",5"-trimethoxyphenyl)ethene

Figure 51 illustrates substituted 3-phosphoramidate salts of combretastatin A-4.

DETAILED DESCRIPTION OF THE INVENTION

We have developed a working hypothesis suggesting that the discovery of new antimitotic agents may result from the judicious combination of a molecular template (scaffold) which in appropriately substituted form (ie. phenolic moieties, etc.) interacts with estrogen receptor (ER), suitably modified with structural features deemed imperative for tubulin binding (arylalkoxy groups, certain halogen substitutions, etc.). The methoxy aryl functionality seems especially important for increased interaction at the colchicine binding site in certain analogs.[16] Upon formulation of this hypothesis concerning ER molecular templates, our initial design and synthesis efforts centered on benzo [*b*]thiophene ligands modeled after raloxifene, the selective estrogen receptor modulator (SERM) developed by Eli Lilly and Co.[17] Our initial studies resulted in the preparation of a very active benzo[*b*]thiophene-based antitubulin agent. [18-21] In further support of our hypothesis, recent studies have shown that certain estrogen receptor (ER) binding compounds as structurally modified estradiol congeners (2-methoxyestradiol, for example) interact with tubulin and inhibit tubulin polymerization.[22] Estradiol is, of course, perhaps the most important estrogen in humans, and it is intriguing and instructive that the addition of the methoxy aryl motif to this compound makes it interactive with tubulin. It is also noteworthy that 2-methoxyestradiol is a natural mammalian metabolite of estradiol and may play a cell growth regulatory role especially prominent during pregnancy.

The design premise that molecular skeletons of traditional estrogen receptor (ER) binding compounds can be modified with structural motifs reminiscent of colchicine and combretastatin A-4 to produce inhibitors of tubulin polymerization has been validated by the benzo[*b*]thiophene and benzol[*b*]furan classes of new antimitotic agents.[18-21] The lead compounds in each series (Figures 1 and 2), demonstrate remarkable biological activity against a variety of human cancer cell lines. For example, the 3,4,5-trimethoxybenzo[*b*]thiophene (Fig. 1) demonstrates potent cytotoxicity and inhibition of tubulin polymerization. In the NCI 60 cell line panel,[23] this compound produces a mean panel $G1_{50} = 2.63 \times 1O^{-7}$ M (see Table I).

Inhibition of tubulin polymerization by 3-(3', 4', 5' - trimethoxybenzoyl)-2-(4'-methoxyphenyl)-6-methoxybenzo[*b*]thiophene. 50% inhibition of the maximum tubulin assembly rate with 1.1 μM drug same assay with conbretastatin A-4 gives a value of 0.73 μM.

Human cancer cell line studies *(in vitro)* by 3-(3', 4', 5' - trimethoxybenzoyl)-2-(4'-methoxyphenyl)-6-methoxybenzo[*b*]thiophene.

Table I. Inhibition of tubulin polymerization by 2-(3', 4', 5'-trimethoxybenzoyl)-3-(4'-methoxyphenyl)-6-methoxybenzo[*b*]furan. IC50 = 2.1 pM (totally flat at 4 pM).

Human cancer cell line studies (*in vitro*) by 2-(3', 4', 5'-trimethoxybenzoyl)-3-(4'-methoxyphenyl)-6-methoxybenzo[*b*]furan.

Type of Cancer Cell Line	Cancer Cell Line	GI_{50} (uglmL)
Pancreas - adn	BXPC-3	0.03 8
Neuroblast	SK-N-SH	0.025
Thyroid ca	SW1736	0.047
Lung-NSC	NCI-H460	0.041
Pharynx-sqam	FADU	0.03 5
Prostate	DU-145	0.062

In addition, the phenolic derivative of the 3,4-5-trimethoxybenzo[*b*]thiophene compound (figure 3) has pronounced cytotoxicity and demonstrates outstanding inhibition of tubulin polymerization[36] and the pro-drug disodium phosphate salt form of this compound (Figure 4) demonstrates *in vitro* and *in vivo* cytotoxicity as a vascular targeting and destruction agent (which includes a component of tubulin binding (phenolic form of drug) [36, 37] and subsequent inhibition of tubulin polymerization).

Initial *in vivo* studies are very encouraging (see Figure 5). Female scid mice were single dose ip administered with CA-4P, and benzo[*b*]thiophene phosphate prodrug at 400mg/kg (i.e. MDT of CA-4P) after one week of MHEC inoculation (1 x 10^{-6}/mouse). Studies were carried out through a collaboration with Professors Ronald W. Pero and Klaus Edvardsen, University of Lund, Sweden (Note: PbT Prodrug 20 is the same compound that is referred to as BBT-P).

Based on these promising research results, our interest in designing an indole based antimitotic agent was initiated, and a synthetic route (Schemes 1-4, see Figures 3A-D) was designed according to the synthesis of the benzo[*b*]thiophene derivatives.

The possibility clearly exists that some of the new indole-based ligands described herein, which are structurally related to combretastatin A-4, may also function through additional biological mechanisms involving anti-angiogenic activity. Clearly the ability to selectively disrupt the blood-flow to developing tumor cells is a potential breakthrough in the ever up-hill

battle against cancer. Certain phenylindoles have been noted for inhibiting tubulin polymerization.[27]

A typical synthesis of indole-based ligand 33 is shown in Figures 6, 9, and 11. Secondary amine 30 was prepared by treatment of *m*-anisidine and 2-bromo-4methoxyacetophenone under basic condition (ethanolic potassium hydroxide) at 0°C. Treatment of amine 30 with PPA resulted in the formation of two regioisomers. These isomers have poor solubility in EtOAc, CH_2Cl_2 and EtOH. Indole 31 was purified (from indole 32) by trituration in acetone. The structure of this isomer was confirmed by NMR analysis. COSY NMR was taken in order to study, in detail, the coupling relationship between the protons. The enlarged COSY spectrum for the aromatic region of ligand 31 is shown in Figure 5. This COSY NMR spectrum, shows a strong coupling between H^a and H^b which each appear as a doublet. H^c is coupled by the proton attached to the nitrogen into a small doublet. H^d is coupled only by H^e into a corresponding doublet, while H^e is coupled both by an ortho coupling (H^d) and by a meta coupling (H^f) into a doublet of doublet pattern. H^f is coupled by H^e into a doublet. Further evidence of the formation of 2-phenyl indole 31 is the chemical shift of the proton H^c on the ring which contains nitrogen. Though computer modeling (ChemDraw Ultra 4.5), the theoretical chemical shift value of 6.4 ppm is predicted for proton H^c (at the 3 position), which matches the peak shown in the actual NMR spectrum at 6.6 ppm. For the case where the proton is at the 2 position (Figure 8), the chemical shift is predicted to be 7.03 ppm, which does not match any peak in the spectrum that was obtained. Based collectively on these studies, the formation of isomer 31 is confirmed, and the migration of the methoxyphenyl system is evidenced. The other isomer (indole 32) is soluble in acetone and is much more difficult to obtain in pure form (see Figure 6).

Alternatively, another synthetic methodology can also be applied to the preparation of the desired 2-phenylindole. In 1984, Angerer and co-workers reported the synthesis of 2-phenylindoles in a one-pot reaction sequence (Figure 9) as a route toward the development of new therapeutic agents for the treatment of endocrine disorders.[25]

Following this procedure (Figure 9), two arylindole regioisomers were obtained in good yield. Recrystallization in EtOH afforded the desired isomer, 2-phenylindole 31, as a white crystalline material.

In order to synthesize the indole-based analog 33, Friedel-Crafts acylation was carried out by treating indole 31 with 3,4,5-trimethoxybenzoyl chloride in the presence of the Lewis-Acid $AlCl_3$ (Figure 10). The reaction did not work under the regular conditions and only

starting material was obtained following work-up. Attempts to modify the reaction conditions by increasing the reaction temperature or using other Lewis Acids, such as $TiCl_4$, proved futile as well. Starting material was recovered in all cases. One possible explanation for this result is the fact that the nitrogen atom (containing a lone pair of electrons and an acidic proton) may disrupt the acylation process. According to this analysis, a Grignard reagent (ethylmagnesium bromide) was used to protect this nitrogen prior to the Friedel-Crafis acylation step. Still, only starting material was obtained following the reaction. Therefore, a new synthetic approach was brought into this study.

In 1977, Inion and co-workers reported the synthesis of a variety of aminoalkoxy4-benzoyl-3-indoles.[26] The benzoate indole product was prepared by treatment of indole with the appropriate benzoyl chloride with heating (130-150°C). HCl is generated under these conditions. A similar synthetic approach was used in the synthesis of the desired trimethoxybenzoate indole ligand 33 (Figure 11).

The precursor, indole 31, was mixed with trimethoxybenzoyl chloride. Since both reagents are solid, a solvent with a high boiling point was needed. 1 ,2-dichlorobenzene was chosen in this case since it has a boiling point of 1 80°C. Under these condition, indole 33 was obtained in moderate yield following purification by flash column chromatography and recrystallization. NMR spectroscopy suggests that the structure of indole 33 is that indicated in Figure 11.

Based on promising results obtained with benzo[*b*]thiophene and benzofuran analogs, the preparation of phosphate salts is detailed in Figures 12-14, the preparation of analogs is detailed in Figures 15-16 and the preparation of similar indole-based phosphate prodrug salts and phosphoramidate derivatives is detailed in Figures 21-51.

In addition to the phosphate ester prodrugs that are described in this application for indole-based anti-mitotic agents, we have also discovered that phosphorous based prodrug derivatives of the nitrogen analog of combretastatin A-4 (CA-4) may have therapeutic advantages as selective tumor vasculature destruction agents. These compounds are primarily phosphoramidate derivatives and related phosphate dianions that are assembled on the 3Æamino substituent of the nitrogen analog of CA-4. Although we describe two specific compounds and several obvious analogs, it should be apparent to anyone skilled in the art, that there are numerous other nitrogen phosphorous bond designs that might be assembled from the

3-amino-combretastatin A-4 structure and that would display similar functionality as prodrugs for the selective destruction of tumor vasculature.

Further significance is given to new drugs that bind to the colchicine site since it has recently been shown that combretastatin CA-4 also demonstrates anti-angiogenesis activity.[24] An emerging area of cancer chemotherapy centers on the development of both anti-angiogenesis drugs which disrupt the new microvessel formation of developing tumors and vascular targeting and destruction agents which selectively target the vasculature of tumor cells while leaving healthy cells intact. Combretastatin CA-4P prodrug (Figure 17A) is one of the leading new candidates from among a relatively small collection of known world compounds which display this vaxcular targeting. Discovered by Professor George R. Pettit (Arizona State University) from a willow tree (*combretum caffrum*) in South Africa in the 1 970s, this compound is currently undergoing phase I clinical evaluation sponsored and licensed by OXiGENE, Inc.

Combretastatin A-4 (CA-4) is a potent inhibitor of tubulin polymerization which binds to the colchicine site on β-tubulin. Interestingly, CA-4 itself does not demonstrate destruction of tumor vasculature, while CA-4 prodrug is very active in terms of tumor vasculature destruction. It is very likely that the phosphate ester portion of the prodrug undergoes dephosphorylation (perhaps through the action of endothelial alkaline phosphatases) selectively at sites of enhanced vascularization to reveal the potent CA-4 itself which destroys the tumor cell through an inhibition of tubulin polymerization. The dephosphorylation event takes place selectively at tumor cells since tumor cells represent sites of prolific vascularization and alkaline phosphatases appear to be present at elevated concentrations in the endothelial cells lining tumor vasculature. This need for enhanced vascularization is not necessary for healthy cells. Hence, this dual-mode reactivity profile is clearly important in order to target tumor cells selectively over healthy cells. This is a proposal which has been advanced by Professor Ronald Pero (OXiGENE, Inc., University of Lund) for which a variety of strong evidence has been obtained.

Based in part on the good and promising biological results obtained for the 3'-nitrogen analogs of combretastatin A-4, a phosphoramidate analog has been prepared as a new combretastatin A-4 nitrogen prodrug (Figure 17B).

Phosphoramidate 10 below was obtained following the procedure reported by Taylor and coworkers for unrelated aryl amines.[28] Treatment of arylamine 7B with diethylchiorophosphite in anhydrous ether followed by oxidation with m-CPBA produced the phosphoramidate 10 in moderate yield (Figure 18).

A previous attempt in the synthesis of the phosphoramidate analog 10 utilized the methodology reported by Bilha Fisher and Larisa Sheihet.[29] This methodology presents a phosphoramidate intermediate, which can be isolated from the reduction of nitro aryl compounds to the corresponding aryl amines using diethylchlorophosphite as a biphilic reagent. The (Z)-nitro combretastatin analog 7B was considered a viable starting material for the synthesis of the phosphoramidate prodrug 10. This reaction was also tried using (Z)-1-(3',4',5'-trimethoxyphenyl)-2-(4"-nitrophenyl)ethene (synthesized in a similar manner as the other combretastatin containing analogs reported previously) as a model system (Figure 19). In neither case was the phosphoramidate product observed. It is thought that the presence of methoxy groups as strong electron donating substituents on the stilbene system disfavors the reaction (Figure 20).

It should be obvious to anyone skilled in the art of phosphate of phosporamidate chemistry that there are numerous other synthetic methods which can be employed to prepare phosphoramidates (such as 10) and their related salts ($-NHPO_3^{-2}2Na^+$).

Table II.[30] *In vitro* Human Cancer Cell Line Study of Phosphoramidate Analog 10. GI_{50}, TGI, and LC_{50} are reported as concentrations in µg/mL ND = Not determined

Cell Type	Cell Line	GI_{50}	TGI	LC_{50}
Pancreas-a	BXPC-3	1.5×10^{-1}	5.7×10^{-1}	>10
Ovarian	OVCAR-3	1.9×10^{-1}	8.6×10^{-1}	>10
CNS	SF-295	2.4×10^{-1}	>10	>10
Lung-NSC	NCI-H460	3.5×10^{-1}	>10	>10
Colon	KM20L2	2.8×10^{-1}	6.1×10^{-1}	>10
Prostate	-DU-145	2.6×10^{-1}	2.6×10^{-1}	>10
Leukemia	P388	3.1×10^{-1}	ND	ND

Biological evaluation (*in vitro*) suggests that the phosphoramidate prodrug 10 is less effective than the corresponding amine 8 (Table II). Pettit and co-workers reported a similar loss in biological activity *in vitro* for the phosphate prodrugs of combretastatin A-4 and phenstatin compared to the original compounds (Table III).[31] These results might be explained by the bulkiness of the phosphorous group and its steric hindrance toward binding site recognition. In

fact, Pettit and co-workers reported no inhibition of tubulin polymerization with the combretastatin prodrug while only a 40% activity is present for the phenstatin prodrug compared to phenstatin. The IC_{50} values for inhibition of tubulin polymerization are 1.2 ± 0.1 μM for CA-4, >80 μM for CA-4 prodrug, 1.0 ± 0.2 μM for phenstatin and 21 ± 3 μM for phenstatin prodrug; similar results are expected for the amino-CA-4 8 and the phosphoramidate 10.[31] The IC_{50} for the amino-CA-4 8 is 1.2 ± 0.02 μM, and the phosphoramidate 10 has little if any activity.[32]

Table III. Comparative GI_{50} Values Against Human Cancer Cell Lines for Amine-CA-4 8, Amine-CA-4 Prodrug 10, Phenstatin, Phenstatin Prodrug and Combretastatin A-4 Prodrug. GI_{50}, values are reported as concentrations in μg/mL ND = Not determined.[a] Data obtained in collaboration with Dr. George R. Pettit.[30b] Data obtained from synthesis of phenstatin phosphate.

Cell Type	Cell-Line	Amine-CA-4 8[a]	Amine-CA-4 Prodrug 10[a]	Phenstatin	Phenstatin Prodrug[b]	Combretastatin A-4 Prodrug[b]
Ovarian	OVCAR-3	ND	1.9×10^{-1}	2.3×10^{-3}	2.5×10^{-3}	2.3×10^{-2}
CNS	SF-295	ND	2.4×10^{-1}	5.2×10^{-2}	1.2×10^{-2}	3.6×10^{-2}
Lung-NSC	NCI-H460	6.8×10^{-4}	3.5×10^{-1}	5.7×10^{-3}	3.5×10^{-2}	2.9×10^{-2}
Colon	KM20L2	ND	2.8×10^{-1}	4.0×10^{-4}	2.7×10^{-1}	3.4×10^{-1}

In terms of *in vivo* systems, phosphoramidate analog 10 is able to provide a more soluble compound than the amine 8, thereby incrementing its bioavailability. Under, *in vivo* biological conditions, the P-N bond can be broken by serum phosphatases releasing the amine which can inhibit tubulin polymerization in a manner analogous to combretastatin

Anti-Angiogenesis

The growth of a tumor depends on the generation of blood vessels which will provide all the metabolites required during cell division. The development of anti-angiogenic compounds is especially useful in the treatment of solid tumors, since these compounds have the potential capability of selectively disrupting the vasculature of tumor cells while leaving healthy cells in a viable situation. The combretastatin A-4 prodrug has demonstrated anti-angiogenic activity since small doses of the drug are toxic to tumor vasculature.[34] Enhanced cytotoxic activity was observed against endothelial cells associated with the tumor vasculature of cancerous cells, while at the same time it was reported to have no effect against other endothelial cells which are

located distant from the tumor itself.[34, 35] The mechanism of action of combretastatin A-4 prodrug, as an anti-angiogenic drug for cancer treatment, is under investigation because the development of blood vessels is crucial for the survival and growth of solid tumors. One proposed mechanism for anti-angiogenesis involves induction of apoptosis (cell suicide) of the cells instead of necrosis. An evaluation of the ability of the new phosphoramidate 10, along with structurally similar compounds, to induce apoptosis of endothelial cells will be undertaken in the near future.

Synthesis of the Phosphoramidate Analog

(Z)-1-(3'-Diethylphosphoramidate-4'-methoxyphenyl)-2-(3", 4", 5"-trimethoxyphenyl)ethene 10.

Diethylchlorophosphite (0.103 g, 0.66 mmol) was dissolved in anhydrous diethyl ether (2.5 ml) and cooled to -78°C. Diisopropylethyl amine (0.187 g. 1.45 mmol) was dissolved in Et_2O (1.0 ml) and added slowly over a period of 2 mm to the reaction mixture by syringe. Amino-stilbene 8 was dissolved in Et_2O (1.0 mL) and added slowly to the reaction mixture by syringe. The reaction mixture was stirred under nitrogen at -78°C for 2 hours, followed by stirring for 1 hour at room temperature. The mixture was filtered, and the solvent was removed under reduced pressure. A yellow oil was obtained which was dissolved in dry CH_2Cl_2 (5 mL). The oil was cooled to -40°C and a solution of *m*-CPBA (0.193 g, 1.12 mmol) in CH_2Cl_2 (5 mL) was added. It was stirred over one hour at room temperature. After this time, the reaction mixture was cooled to -40°C and filtered through a sintered glass funnel. The liquid was collected with vigorous stirring over sodium sulfite (5%) (20 ml) in order to quench the reaction. The product was isolated by extraction with CH_2Cl_2, and washed with a saturated solution of $NaHCO_3$. The yellow oil which was obtained was dried over $MgSO_4$. Purification by flash chromatography (70/30, hexanes/EtOAc) afforded the phosphoramidate 10 as a yellow oil (0.130 g, 0.29 mmol, 44%).

'H-NMR ($CDCl_3$, 360 MHz) δ 7.12 (d,J= 1.9 Hz, IH, Ar*H*), 6.88 (dd, J= 8.4 Hz, 2.0 Hz, I*H*, Ar*H*), 6.72 (dd, J= 8.4 Hz, 1.7, IH, Ar*H*), 6.49 (s, 2H, Ar*H*), 6.51 (d, J= 12.1 Hz, IH, vinyl C*H*), 6.41 (d, J= 12.1 Hz, IH,vinyl C*H*), 5.67 (d, J= 10.0 Hz, N*H*), 4.02 (m, 4H, CH_2), 3.83 (s, 3 H, OCH_3), 3.83 (s, 3 H, OC_3), 3.68 (s, 6 H, OCH_3), 1.25 (t, 6 H, J= 7.1 Hz, CH_3).

^{13}C-NMR ($CDCl_3$, 90 MHz) δ 152.7, 146.7, 146.6, 137.0, 132.7, 130.3, 129.7, 129.1, 129.0, 122.0, 117.0, 109.9, 106.0, 62.8, 60.7, 55.7, 16.1.

^{31}P-NMR ($CDCl_3$, 145 MHz) δ 0.84.
HRMS (EI) M+ calcd for $C_{22}H_{30}N0_7P$ 451.1760, found 451.1765.

Example 1
SYNTHESIS OF THE INDOLE-BASED ANTI-TUBULIN AGENTS

Preparation of 2-Phenyl Indole 31
Method I (2 steps):

To a well-stirred solution of KOH (0.926 g, 16.5 mmol) in EtOH (18 ml) and H_20 (9 ml) at rt was added *m*-anisidine (2.192 g, 17.80 mmol) by syringe. The solution was then stirred at 0°C. After 10 min, the solution of 2-bromo-4-methoxyacetophenone (4.09 g, 17.80 mmol) was added dropwise with an addition funnel over a 40 minute period. After 24 h, 0°C to rt, water was added. The product was isolated by extraction (I H HCI, NaHCO3, brine, MgSO4). The product was purified by recrystallization (50:50 EtOAc:hexanes) to afford secondary amine 30 (2.46 g, 9.07 mmol, 52%) as yellow solid.

'H NMR ($CDCl_3$): δ 7.98 (2H, D, J = 8.9 Hz), 7.12 (IH, t, J 8.1 Hz), 6.97 (2H, d, J 8.9 Hz), 6.30 (3H, m), 4.54 (2H, s), 3.88 (3H, s), 3.79 (3H, s).

Polyphosphoric acid (PPA) was charged to a round-bottom flask and the temperature was raised to 80°C with vigirous stirring. To this flask was added the foregoing amine 30 (4.0 g, 14.74 mmol) in 6 portions over a 30 minute period. After 2 h, 80°C to 90°C, water was added. The product was isolated by extraction (EtOAc, $NaHCO_3$, brine, $MgSO_4$). Purification by recrystallization (acetone) afforded indole 31(0.544 g, 2.15 mmol, 15%) as a pale yellow solid.

'H NMR ($CDCl_3$): δ 11.24 (IH, br, s), 7.72 (2H, d, J 8.82 Hz), 7.36 (IH, d, J = 8.57 Hz), 7.00 (2H, d, J= 8.84 Hz), 6.85 (IH, d, J = 2.07 Hz), 6.66 (IH, d, J = 1.66 Hz), 6.63 (IH, dd, J 8.59, 2.28 Hz), 3.78 (3H, s), 3.77 (3H, s).

^{13}C NMR ($CDCl_3$): δ 158.15, 155.22, 137.44, 136.33, 125.60, 124.93, 122.82, 120.04, 114.07, 109.00, 96.97, 94.01, 54.93, 54.88.

Method 2(1 step):

To a boiling mixture of in-anisidine (1.56 ml, 20.0 minol) and *N,N*-dimethylaniline (3.5 ml) was added 2-bromo-4-methoxyacetophenone (1.37 g in EtOAc, 6.00 mmol) slowly by syringe. After addition, the mixture was kept at 170° C for 1 hour. The reaction mixture was cooled to room temperature and a dark colored solid was formed. EtOAc was added along with HCI (2 N). The aqueous layer was extracted with EtOAc several times. The combined organic layers were washed with brine, and dried over $MgSO_4$. Solvent was removed under the reduced pressure to afford a dark brown colored solid. Purification by recrystallization in EtOH afforded indole 31 as a white crystalline material.

'H NMR($CDCl_3$): δ 11.24 (IH,br,s),7.72(2H,d, *J* 8.82 Hz), 7.36 (IH,d, *J* 8.57 Hz),7.00 (2H,d, *J* = 8.84 Hz), 6.85 (IH, d, *J*= 2.07 Hz), 6.66 (IH, d, *J*= 1.66 Hz), 6.63 (IH, dd, *J* 8.59, 2.28 Hz),3.78 (3H, s), 3.77 (3H, s).

^{13}C NMR ($CDCl_3$): δ 158.15, 155.22, 137.44, 136.33, 125.60, 124.93, 122.82, 120.04, 114.07, 109.00, 96.97, 94.01, 54.93, 54.88.

Melting Point: 208-229.5°C

HRMS (El) M+ calcd for $CH_{16}N0_2$ 253.3035, found 253.1060.

Preparation of Trimethoxybenzoate 2-Phenylindole 33

To a well stirred solution of indole 31(0.502 g, 1.98 mmol) in *o*.dichlorobenzene (10 ml) was added trimethoxybenzoylchloride (0.692 g, 3.00 mmol). The reaction mixture was heated to reflux for 12 hours. Solvent was removed by distillation under reduced pressure. After cooling down to room temperature, a dark solid formed which was dissolved in chloroform and purified by silica gel column chromatography with chloroform as the eluent. The collected mixture was again purified by column chromatography (50:50 hexanes:EtOAc) affording trimethoxybenzyl

indole 33 (0.744 g, 1.66 mmol, 84%) as a yellow oily gel. Pale yellow-green crystals were obtained by recrystallization from a mixture of ethanol and hexanes.

'H NMR ($CDCl_3$): δ 8.63 (IH, br, s), 7.88 (IH, d, J = 9.39 Hz), 7.24 (2H, d, J= 8.78 Hz), 6.95(2H, s), 6.90 (2H, m), 6.71 (2H, d, J= 8.79 Hz), 3.86 (3H, s), 3.80 (3H, s), 3.73 (3H, s), 3.68 (6H, s);

^{13}C NMR ($CDCl_3$): δ 192.23, 159.73, 157.06, 152.42, 142.85, 141.01, 136.41, 134.65, 130.16, 124.28, 122.94, 122.17, 113.67, 112.46, 111.52, 107.24, 94.54, 60.78, 55.92, 55.54, 55.14.

Melting Point: 153-155°C

Anal. Calcd for $C_{26}H_{25}N0_6$: C, 69.79; H, 5.63; H, 3.13. Found: C, 69.61; H, 5.63; N, 3.01.

EXAMPLE 2

INHIBITION OF TUBULIN POLYMERIZATION ASSAY

IC_{50} values for tubulin polymerization were determined according to the procedure described in Bai et al. Purified tubulin is obtained from bovine brain cells as described in Hamel and Lin. Various amounts of inhibitor were preincubated for 15 minutes at 37° C with purified tubulin. After the incubation period, the reaction was cooled and GTP was added to induce tubulin polymerization. Polymerization was then monitored in a Gilford spectrophotometer at 350 nm. The final reaction mixtures (0.25 ml) contained 1.5 mg/ml tubulin, 0.6 mg/ml microtubule-associated proteins (MAPs), 0.5 mM GTP, 0.5 mlM $MgCl_2$, 4% DMSO and 0.1M 4-morpholineethanesulfonate buffer (MES, pH 6.4). IC_{50} is the amount of inhibitor needed to inhibit tubulin polymerization 50% with respect to the amount of inhibition that occurs in the absence of inhibitor. The IC_{50} value determined for 3 -(3' ,4' ,5' -trimethoxybenzoyl)-2-(4' -methoxyphenyl)-6-methoxyindole was 0.5-1.5 μM.

EXAMPLE 3

CYTOTOXIC ASSAY WITH P388 LEUKEMIA CELLS

One of the newly prepared compounds was evaluated for cytotoxic activity against P388 leukemia cells using an assay system similar to the National Cancer Institute procedure described below and in Monks et al. The ED50 value (defined as the effective dosage required to inhibit 50% of cell growth) of 3-(3',4',5' trimethoxybenzoyl)-2-(4'-methoxyphenyl)-6-methoxyindole was found to be 0.0133 μg/mL.

EXAMPLE 4

GROWTH INHIBITORY ACTIVITY AGAINST OTHER CANCER CELL LINES

3 -(3' ,4' ,5'-Trimethoxybenzoyl)-2-(4' -methoxyphenyl)-6-methoxyindole was evaluated in terms of growth inhibitory activity against several human cancer cell lines, including pancreas, ovarian, CNS, lung-NSC, colon, and prostate lines. The assay used is described in Monks et al. Briefly, the cell suspensions, diluted according to the particular cell type and the expected target cell density (5,000-40,000 cells per well based on cell growth characteristics), were added by pipet (100 μl) to 96-well microtiter plates. Inoculates were allowed a preincubation time of 24-28 hours at 37°C for stabilization. Incubation with the inhibitor compounds lasted for 48 hours in 5% CO_2 atmosphere and 100% humidity. Determination of cell growth was done by in situ fixation of cells, followed by staining with a protein-binding dye, sulforhodamine B (SRB), which binds to the basic amino acids of cellular macromolecules. The solubilized stain was measured spectrophotometrically. The results of these assays are shown in Table 1. $G1_{50}$ is defined as the dosage required to inhibit tumor cell growth by 50%.

Table IV. Activity of Indole Ligand Against Selected Human Cancer Cell lines (*In Vitro*).

		Indole-based Ligand 33
CELL TYPE	CELL LINE	GI_{50} (μG/mL)
Pancreas-a	BXPC-3	2.0×10^{-3}
Ovarian	OVCAR-3	2.4×10^{-3}
CNS	SF-295	2.4×10^{-3}
Lung-NSC	NCI-H460	2.6×10^{-3}
Colon	KM20L2	1.7×10^{-3}
Prostate	DU-145	2.3×10^{-3}

Indole and indole containing compounds of therapeutic efficacy have been known for many, many years. What is truly unique about the indole compounds described in this application is the fact that these compounds are the first (to the best of our knowledge) indole-based ligands to incorporate the 3,4,5-trimethoxyaryl motif reminiscent of colchicine and combretastatin A-4 arranged in an appropriate molecular conformation such that a pseudo aryl-aryl pi stacking interaction can take place. It is our contention that such an aryl-aryl interaction of the appropriate centroid-to-centroid distance (approximately 4.7 Å) is imperative for enhanced binding affinity to the colchicine site on β-tubulin. It is this binding that ultimately leads to an inhibition of tubulin polymerization which manifests itself as a cytotoxic event. It should be readily apparent to any practitioner skilled in the art that there are various ways of appending trimethoxyaryl and trimethoxyaroyl groups around an indole molecular scaffold in a manner which will result in a similar molecular conformation capable of undergoing pseudo pi-pi stacking. In addition, although the trimethoxyaryl motif seems optimal for enhanced tubulin binding, it is also very possible that another combination of alkoxy substituents (such as ethoxy, propoxy, isopropoxy, allyloxy, etc.) either as a trisubstituted pattern or as disubstituted (with one type of alkoxy moiety) and monosubstituted (with a different alkoxy moiety), or with three distinct types of alkoxy moieties may also have good tubulin binding characteristics. It is also conceivable that instead of having aryl alkoxy groups, it may be possible to substitute simply aryl-alkyl and aryl-alkenyl moieties and still maintaln the enhanced cytotoxicity profile. Phenolic groups may also have activity on these described indole ligands. The synthesis of any of these modified

indole-ligands will be very straight-forward for anyone skilled in the art, and often will only involve a different choice of initial starting materials. To prepare these alternative ligands, the same synthetic schemes (Figures 6, 9, 11, 12-16), or similar schemes with only slight modifications may be employed. In previous studies with the benzo[*b*]thiophene ligands, we have demonstrated that the carbonyl group can be replaced with an oxygen to generate a new compound which maintains the same or similar biological efficacy with tubulin. Similarly, the replacement of the carbonyl group in the described indole ligand may be replaced with an oxygen atom (ether linkage) to generate a new derivative which would be predicted to have good activity with tubulin. This compound may be prepared by an addition elimination reaction utilizing the trimethoxyphenolic anion as a nucleophile as described by us for the benzo[*b*]thiophene compounds. Other linkage atoms between the aryl aryl rings are conceivable as well.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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32) Tubulin polymerization studies carried out through collaborative efforts with Dr Ernest Hamel, National Institute of Health, National Cancer Institute.

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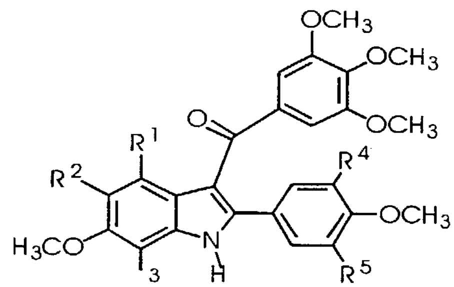
What is claimed is:

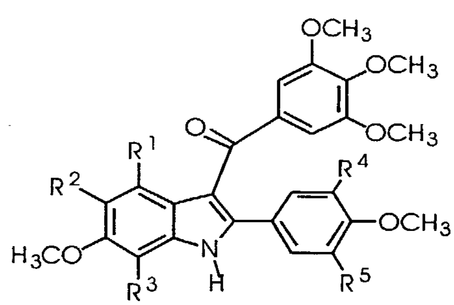
1. A compound of the structure:



wherein

R^1 through R^5 contain at least one phenolic moiety or at least one amine group (NH_2, NHR^1, or NR^6R^7 where R^6 and R^7 are the same or different alkyl having up to 8 carbon atoms), benzyl, or aryl while the remaining R^1 through R^5 are hydrogen.

2. A compound of the structure:

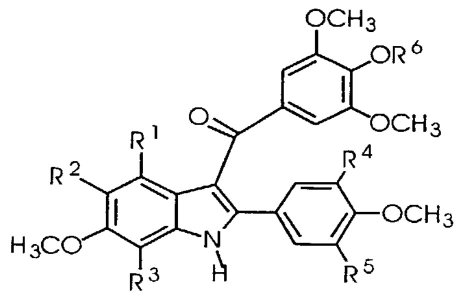


wherein

R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where

R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different, benzyl, or aryl while the remaining R^1 through R^5 are hydrogen.

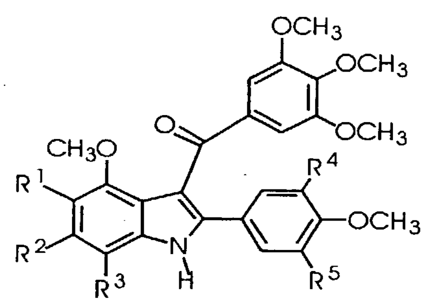
3. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl while the remaining R^1 through R^5 are hydrogen, and R^6 is hydrogen or alkyl.

4. A compound of the structure:

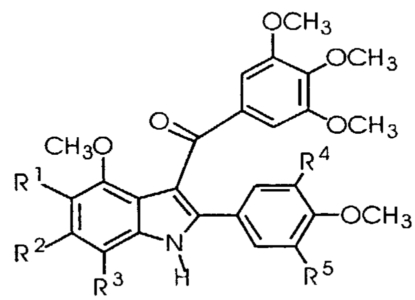


wherein

R^1 through R^5 contain at least one phenolic moiety or at least one amine (NH_2, NHR^1, or NR^6R^7 where R^6 and R^7 the same or different alkyl having up to 8

carbon atoms, benzyl, or aryl groups) while the remaining R^1 through R^5 are a hydrogen.

5. A compound of the structure:



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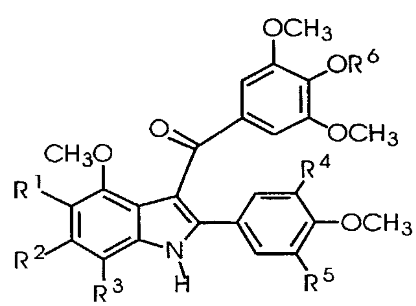
R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl while the remaining R^1 through R^5 are hydrogen.

6. A compound of the structure:

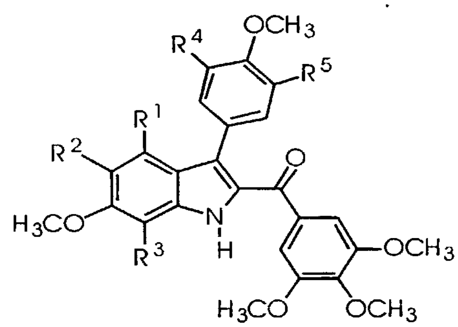


wherein

R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M = a cation or ($-NP(O)(OR)_2$) where

R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), or benzyl, or aryl groups, while the remaining R^1 through R^5 are a hydrogen and R^6 is hydrogen or alkyl.

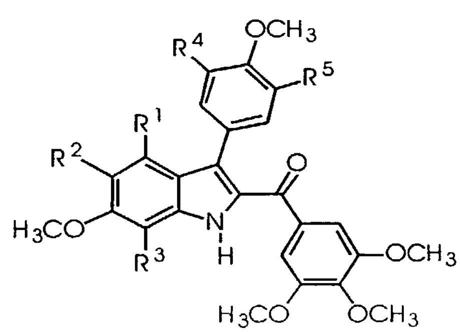
7. A compound of the structure:



wherein

R^1 through R^5 contain at least one phenolic moiety or at least one amine group (NH_2, NHRor NR^6R^7 where R^6 and R^7 are the same or different alkyl having up to 8 carbon atoms may be the same or different), or benzyl, or aryl groups) while the remaining R^1 through R^5 are a hydrogen.

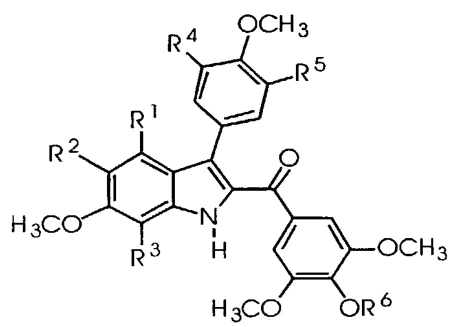
8. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl while the remaining R^1 through R^5 are hydrogen.

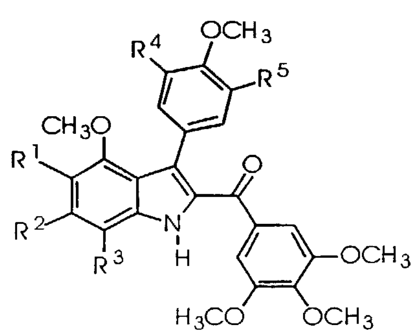
9. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ($-OP(O)(O^-M^+)_2$) or phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl, while the remaining R^1 through R^5 are hydrogen, and R^6 is hydrogen or alkyl.

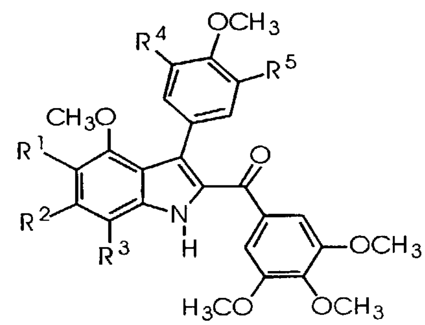
10. A compound of the structure:



wherein

R^1 through R^5 contain at least one phenolic moiety or at least one amine group (NH_2, NHR^1, or NR^6R^7 where R^6 and R^7 are the same or different alkyl having upt to 8 carbon atoms, benzyl, or aryl) while the remaining R^1 through R^5 are a hydrogen.

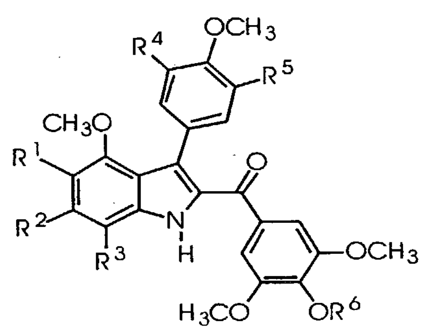
11. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl, while the remaining R^1 through R^5 are hydrogen.

12. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an

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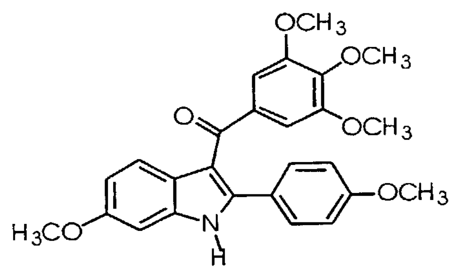
13. A compound of the structure:



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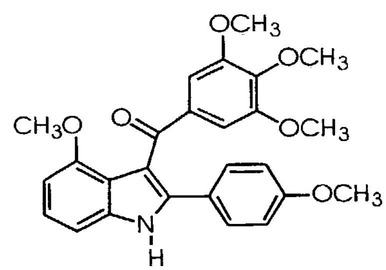

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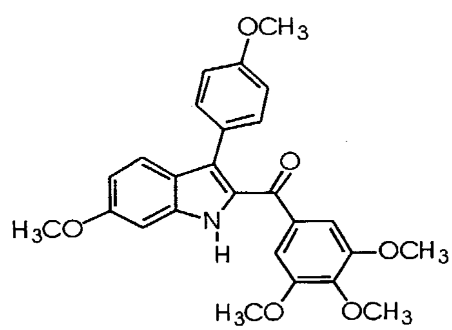

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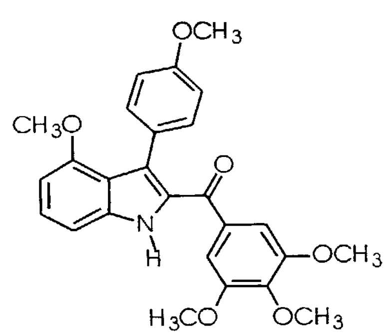

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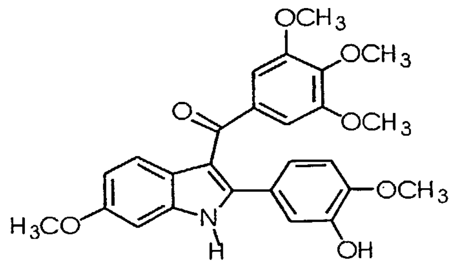

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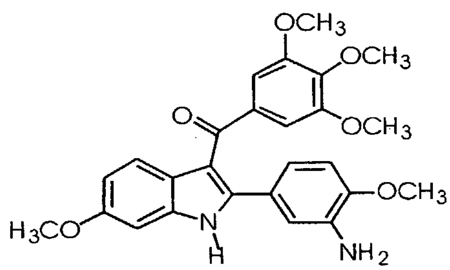

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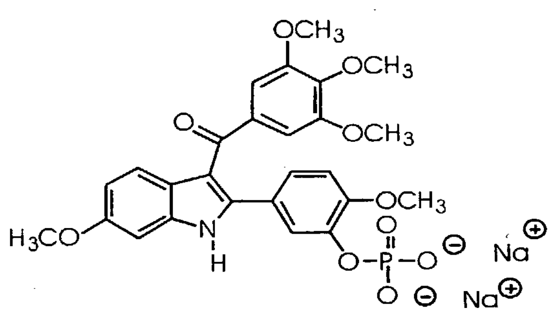

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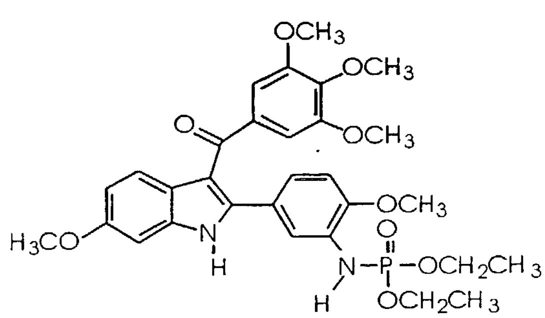

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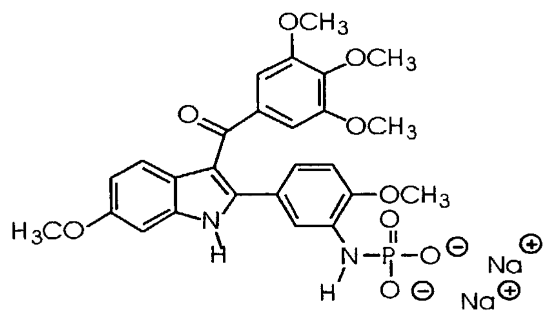

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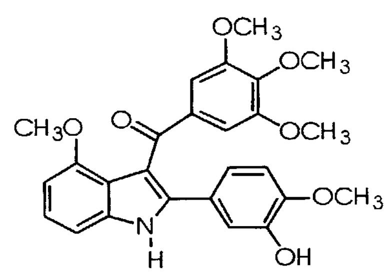

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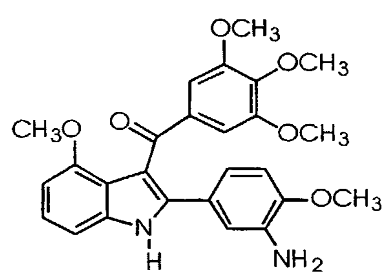

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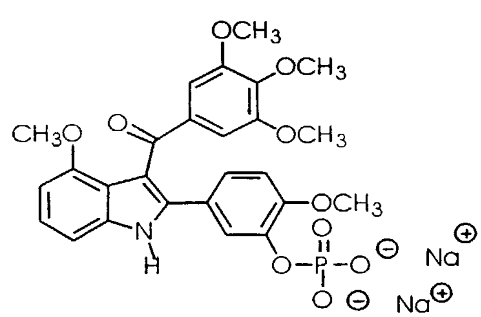

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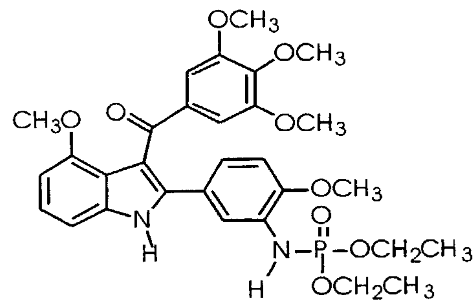

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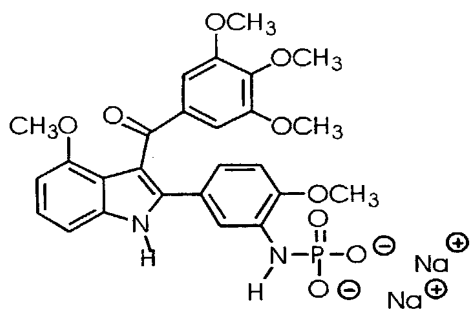

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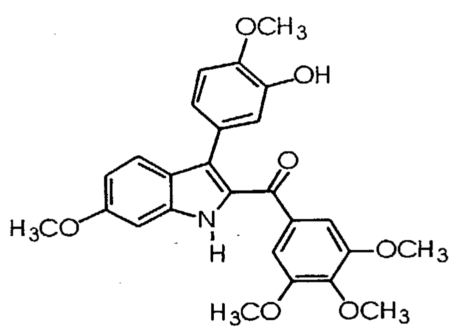

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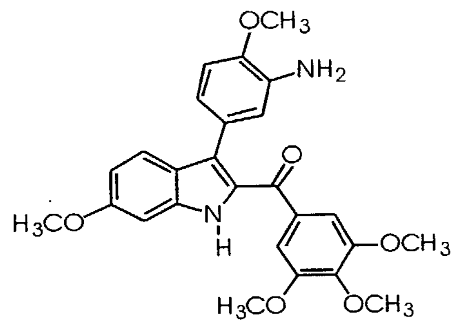

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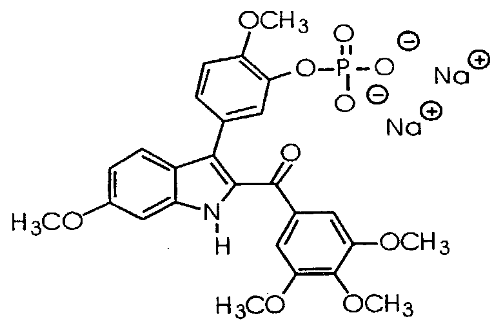

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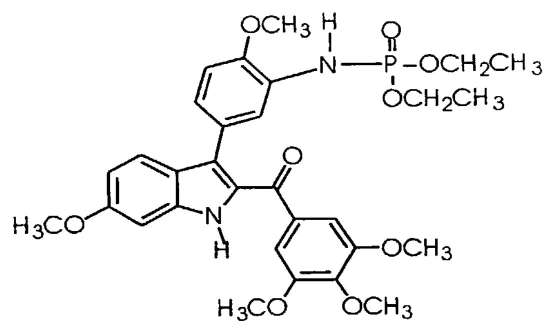

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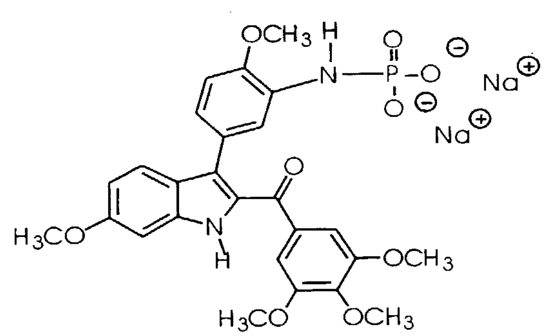

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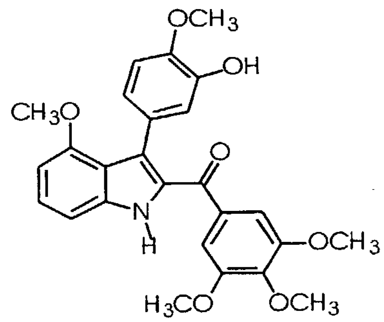

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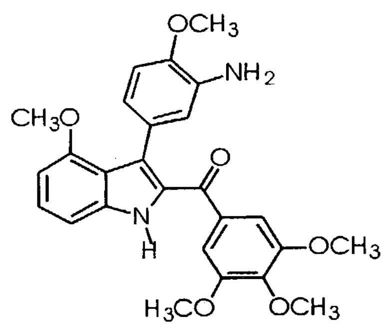

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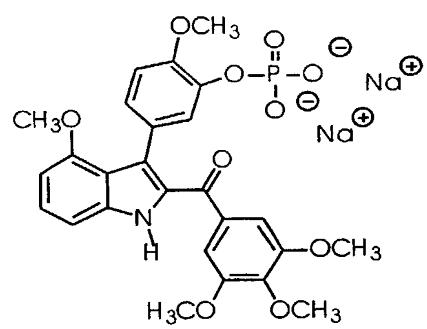

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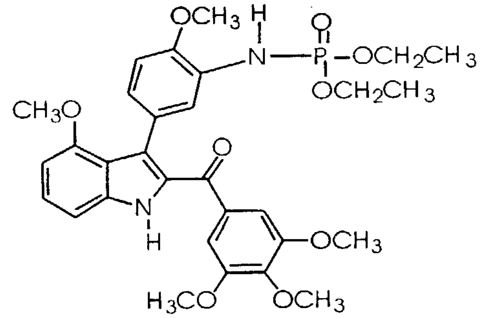

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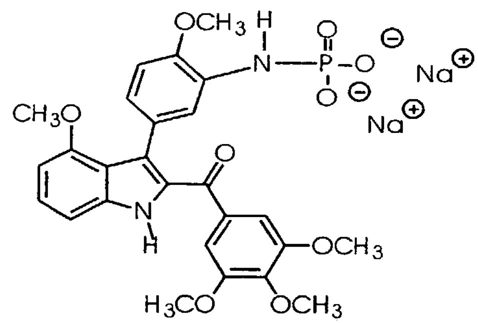

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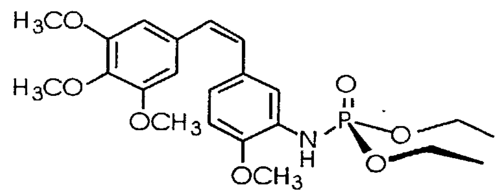

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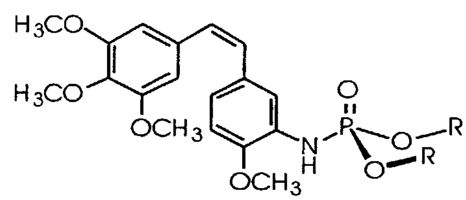
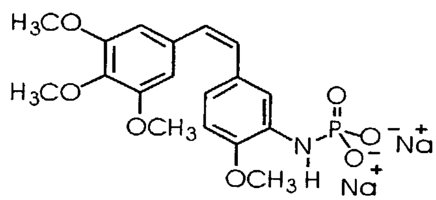


wherein

R is chosen to be any appropriate alkyl or branched alkyl having up to 8 carbon atoms, the two R groups may be the same or different.

39. A compound of the structure:



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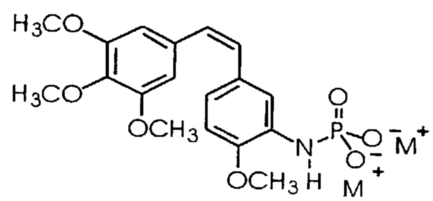

wherein

M^+ is a cation.

41. A method for inhibitiing tubulin polymerization by contacting a tubulin-containing system with an effective amount of a compound described in any of claims 1-40.

42. The method of claim 41 wherein said system is in a tumor cell.

43. A method of treating a host afflicted with a neoplastic disease by administering to said host a compound described in any of claims 1-40.

44. The method of claims 41, wherein the contacted system is located in a patient.

45. The method of claim 41 described further as for treating cancer, wherein said cancer may be chosen from the group containing leukemia, lung, colon, thyroid, CNS, melanoma, ovarian, renal, prostate, and breast cancers.

46. A preparation for pharmaceutical use containing a compound from any of claims 1-40 as an active component along with a pharmaceutically acceptable carrier.

47. A method for selectively targeting and destroying tumor vasculature comprising administering an effective amount of a compound described in any of claims 1-40.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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PCT

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(51) International Patent Classification[7]: C07D 209/12, A61K 31/404, A61P 43/00, C07F 9/572, 9/24

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(74) Agent: **HODGINS, Daniel, S.**; Head, Johnson & Kachigian, 228 West 17th Place, Tulsa, OK 74119 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 27 September 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/19794 A3

(54) Title: INDOLE-CONTAINING AND COMBRETASTATIN-RELATED ANTI-MITOTIC AND ANTI-TUBULIN POLYMERIZATION AGENTS

(57) Abstract: Trimethoxyphenyl substituted indole ligands have been discovered which demonstrate impressive cytotoxicity as well as a remarkable ability to inhibit tubulin polymerization. Such compounds as well as related derivatives are excellent clinical candidates for the treatment of cancer in humans. In addition, certain of these ligands, as pro-drugs, may well prove to be tumor selective vascular targeting and destruction chemotherapeutic agents or to have anti-angiogenesis activity resulting in the selective prevention and/or destruction of tumor cell vasculature.

FIG. 1

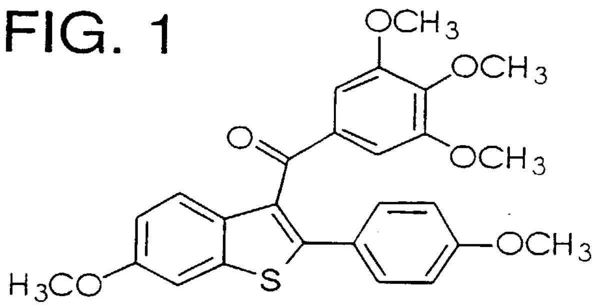

FIG. 2

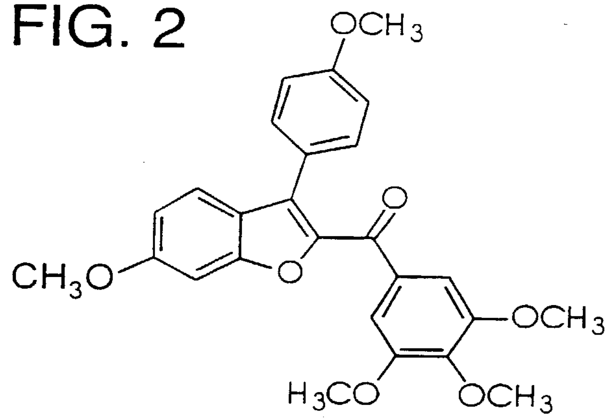

FIG. 3

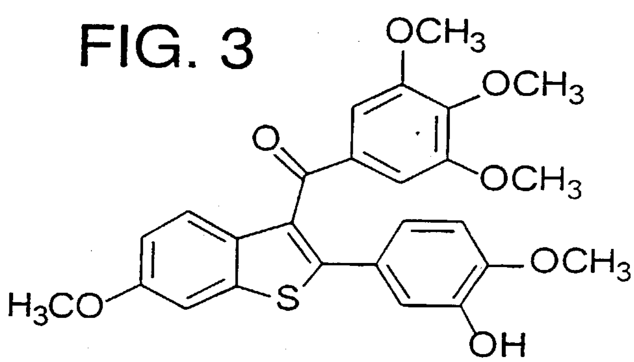

FIG. 4

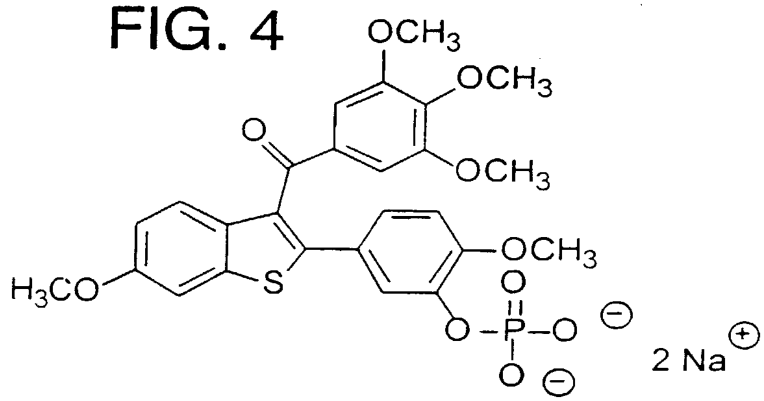

FIG. 5

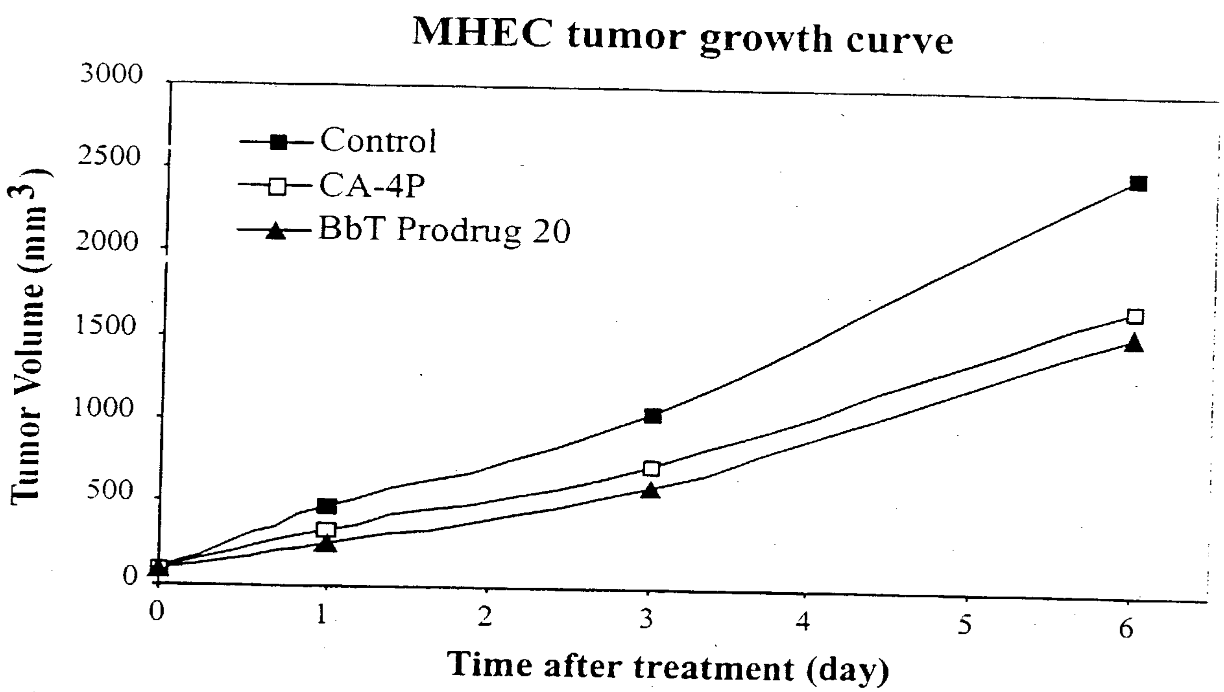
MHEC tumor growth curve
Control
CA-4P
BbT Prodrug 20
Tumor Volume (mm3)
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
5
6
Time after treatment (day)

FIG. 6

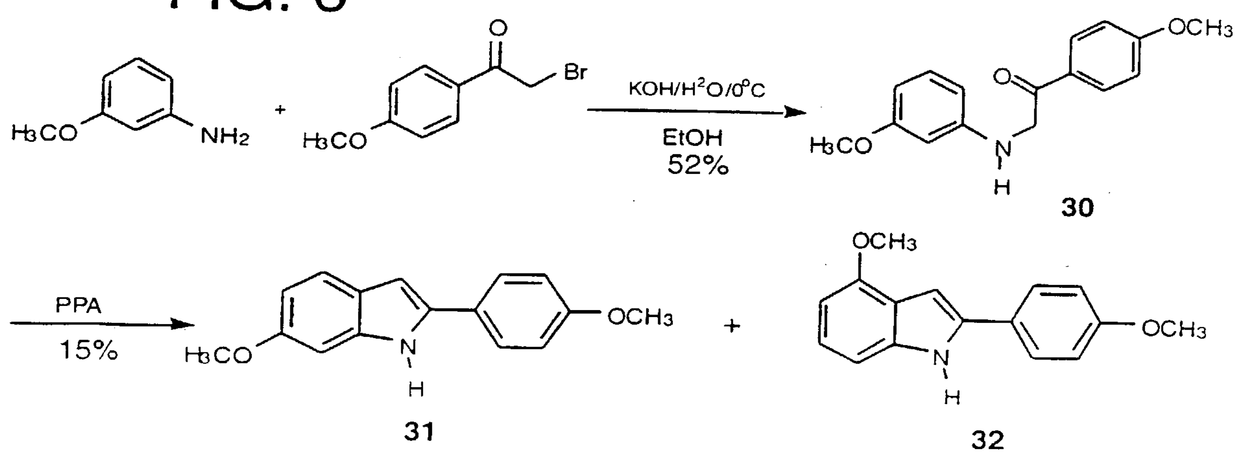
H3CO
NH2
+
O
Br
H3CO
KOH/H2O/0°C
EtOH
52%
OCH3
O
H3CO
N
H
30
PPA
15%
H3CO
N
H
OCH3
31
+
OCH3
N
H
OCH3
32

FIG. 7

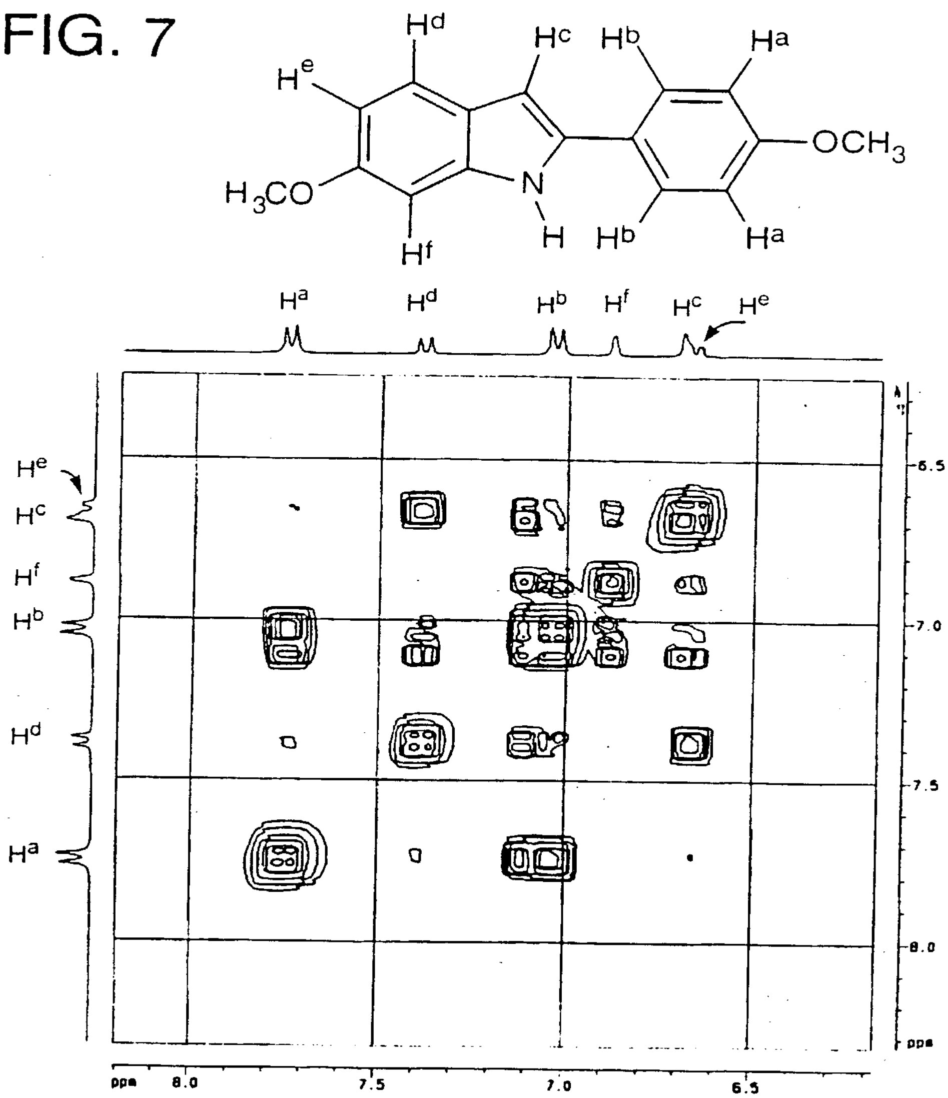
Hd
Hc
Hb
Ha
He
OCH3
H3CO
N
H
Hf
ppm
8.0
7.5
7.0
6.5

FIG. 8

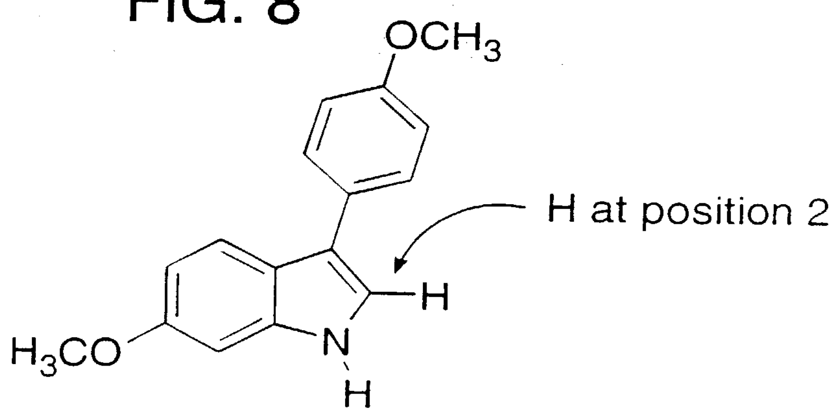
OCH3
H at position 2
H
H3CO
N
H

FIG. 9

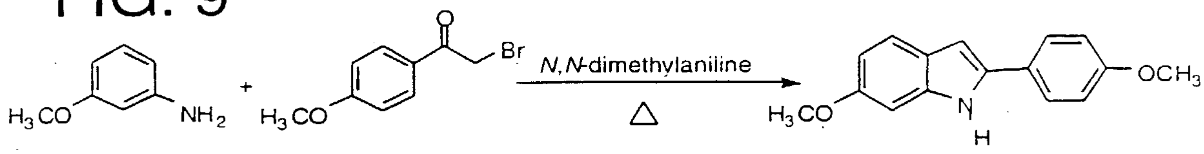

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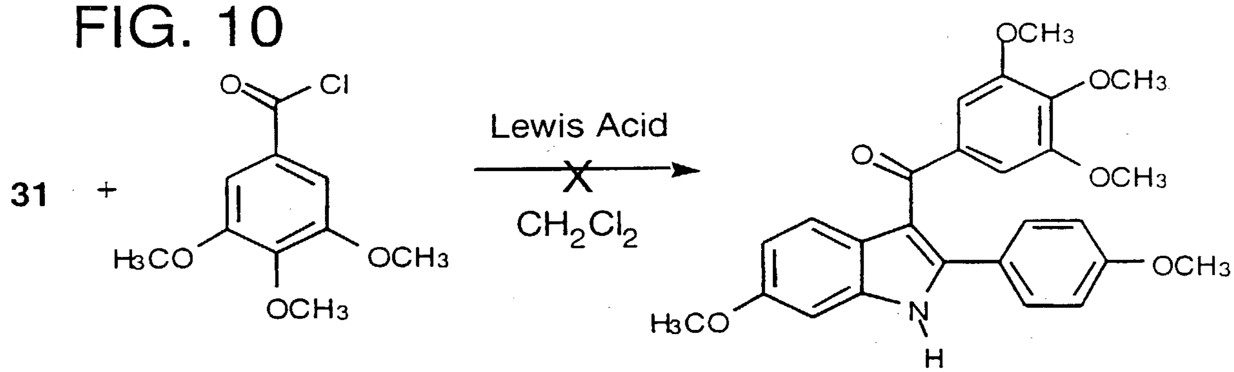
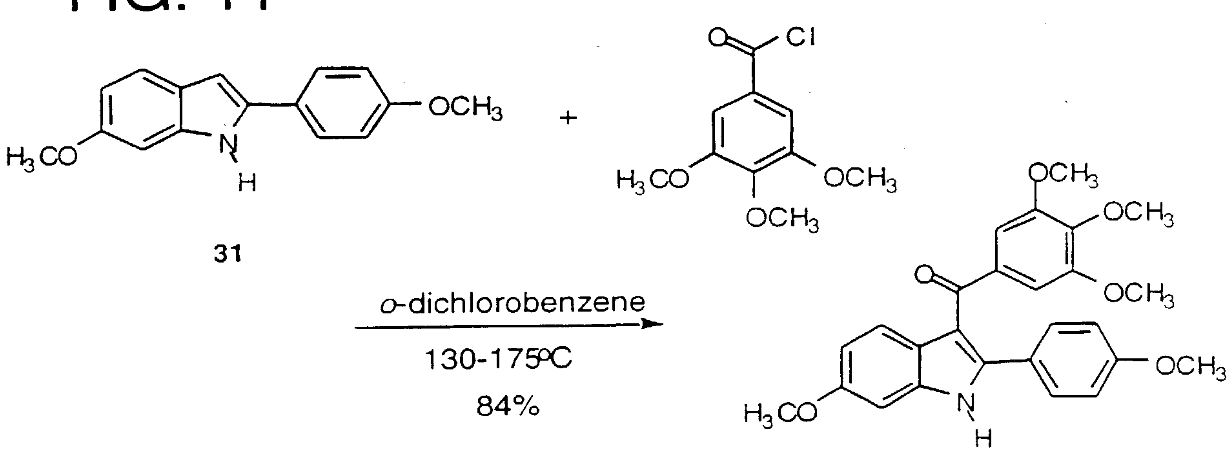
FIG. 10



33

FIG. 11



33

FIG. 12

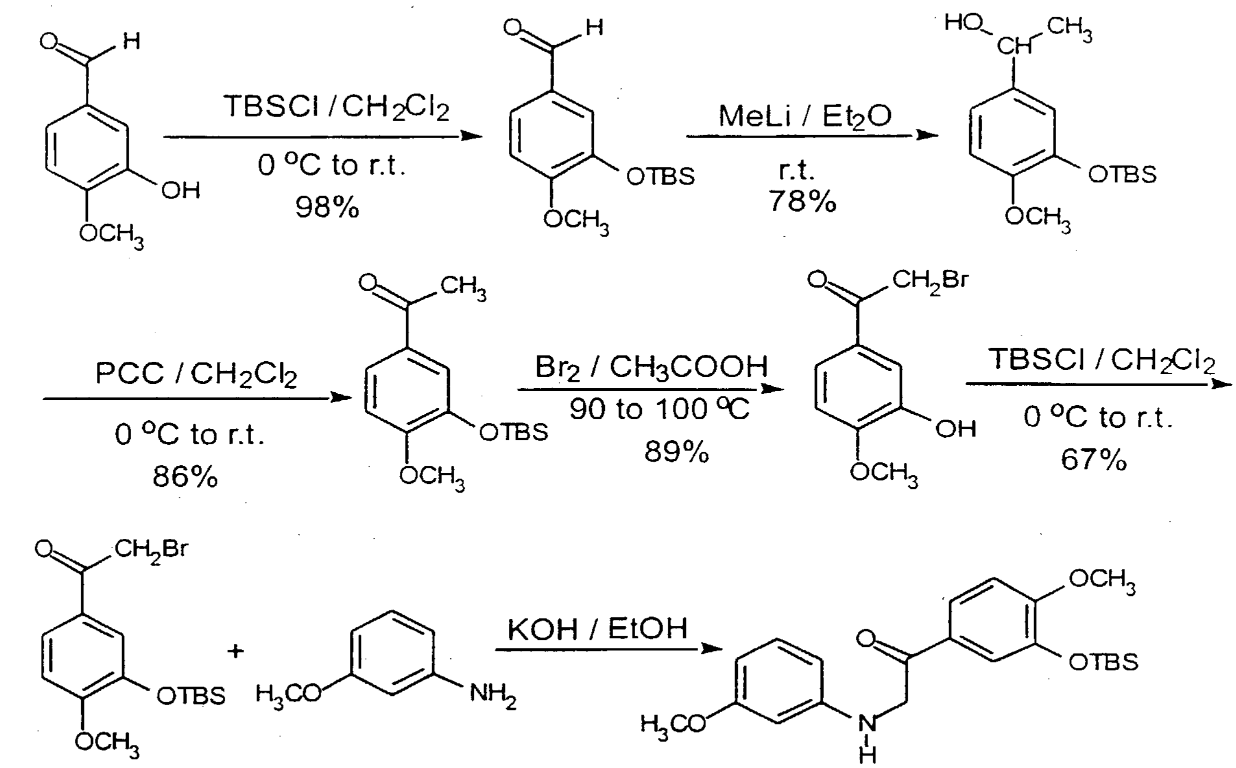

TBSCl / CH_2Cl_2
0 °C to r.t.
98%
OTBS
MeLi / Et_2O
r.t.
78%
PCC / CH_2Cl_2
0 °C to r.t.
86%
Br_2 / CH_3COOH
90 to 100 °C
89%
TBSCl / CH_2Cl_2
0 °C to r.t.
67%
KOH / EtOH

FIG. 13

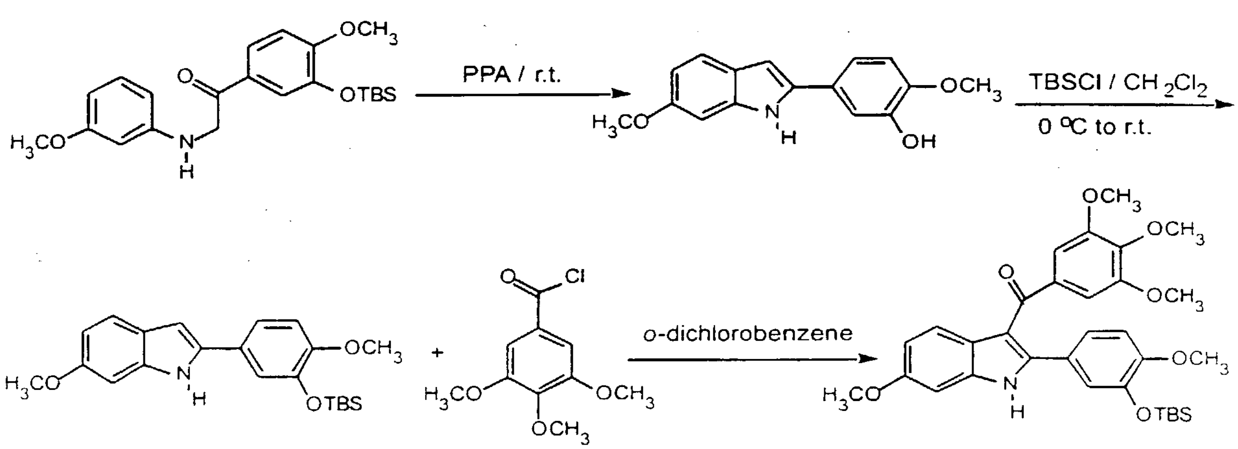

PPA / r.t.
TBSCl / CH_2Cl_2
0 °C to r.t.
o-dichlorobenzene

FIG. 14

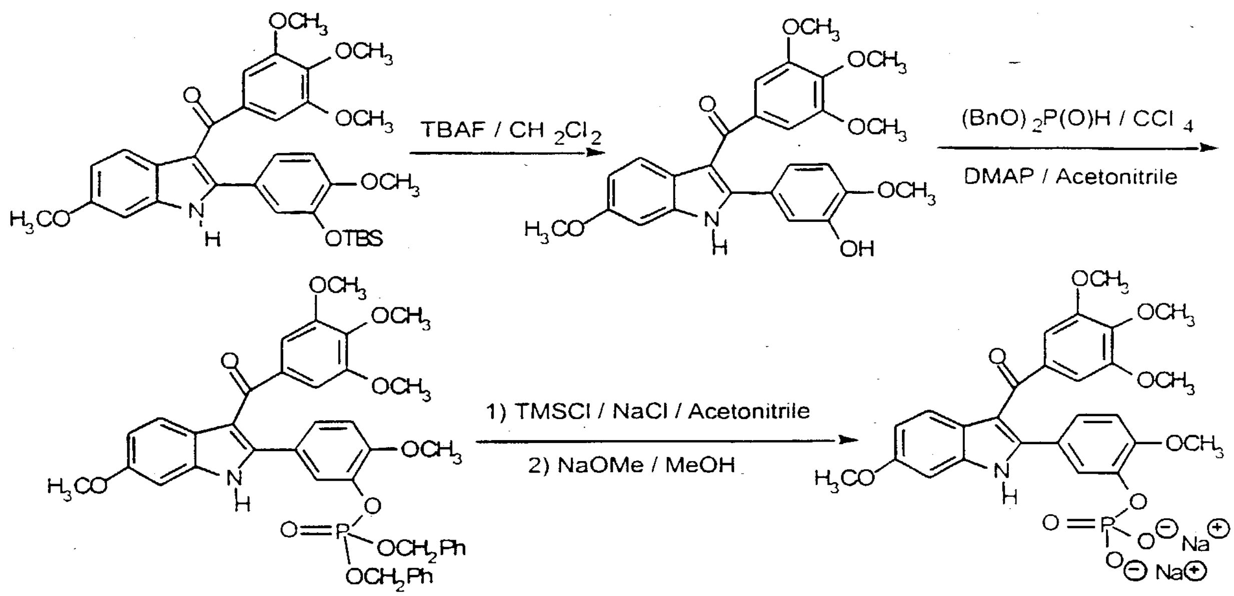

TBAF / CH 2Cl2
(BnO) 2P(O)H / CCl 4
DMAP / Acetonitrile
1) TMSCl / NaCl / Acetonitrile
2) NaOMe / MeOH

FIG. 15

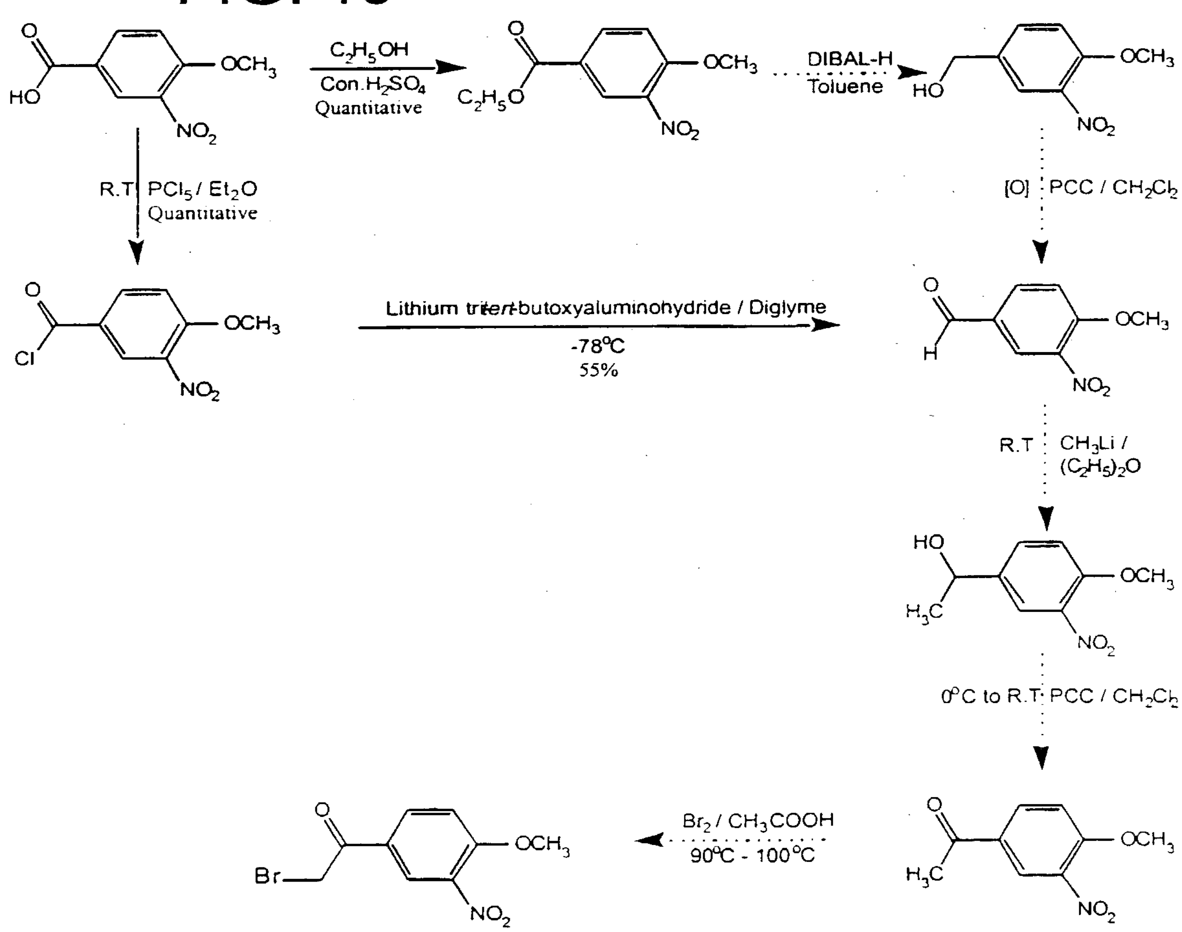

C2H5OH
Con.H2SO4
Quantitative
DIBAL-H
Toluene
R.T PCl5 / Et2O
Quantitative
[O] PCC / CH2Cl2
Lithium tri-tert-butoxyaluminohydride / Diglyme
-78°C
55%
R.T CH3Li / (C2H5)2O
0°C to R.T PCC / CH2Cl2
Br2 / CH3COOH
90°C - 100°C

FIG. 16

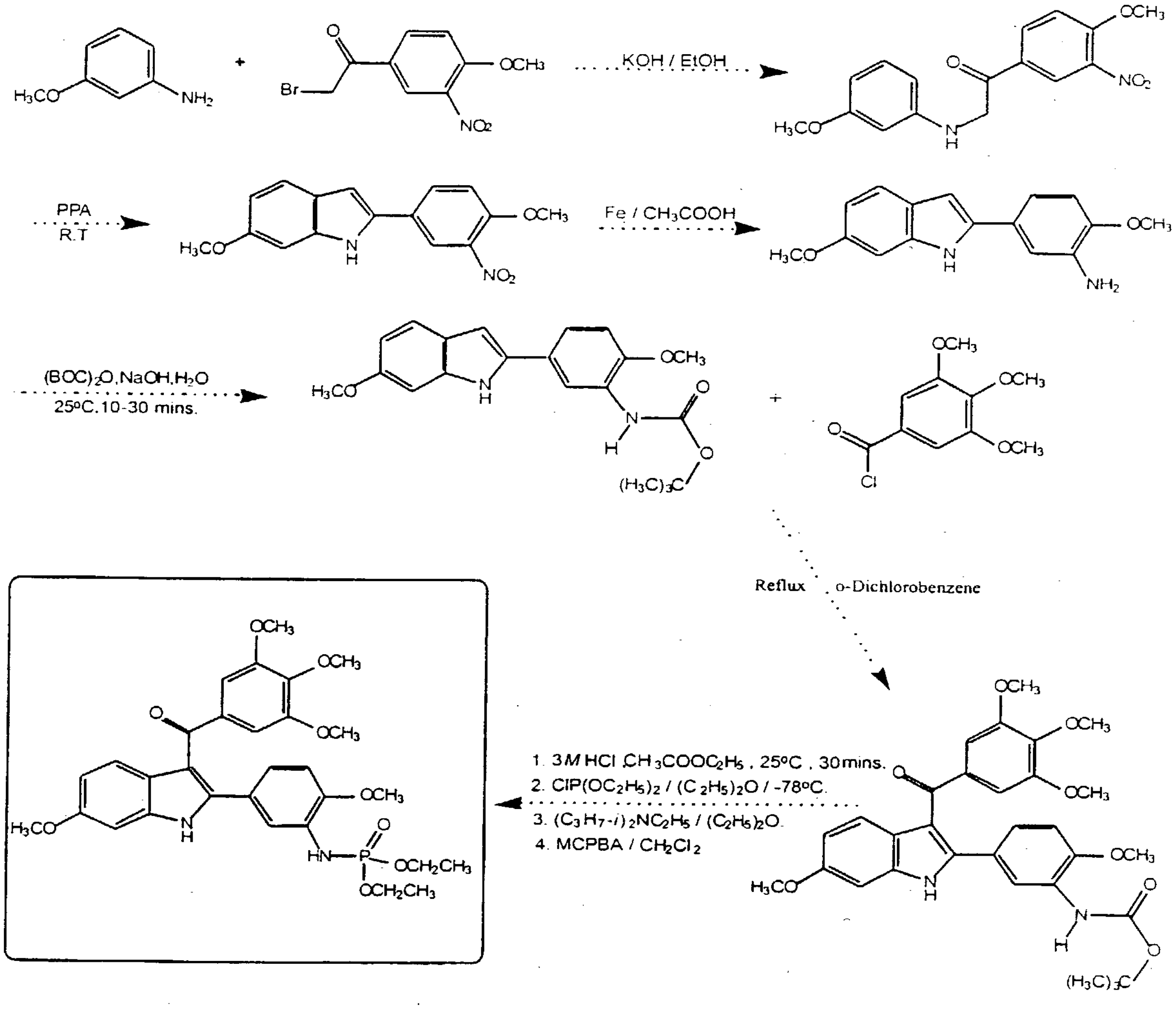


FIG. 17A

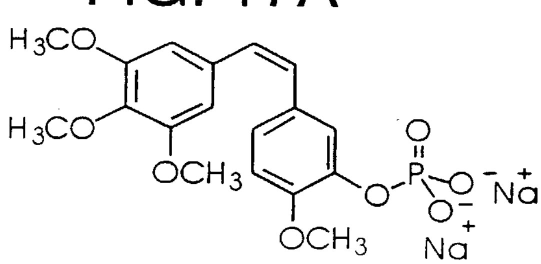


FIG. 17B

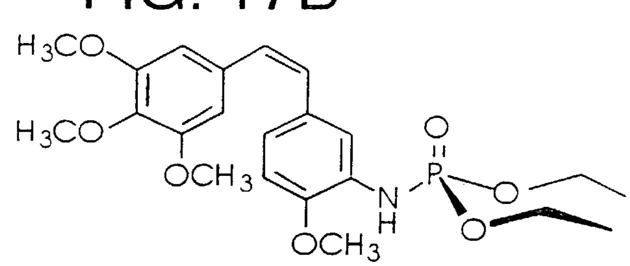


FIG. 18

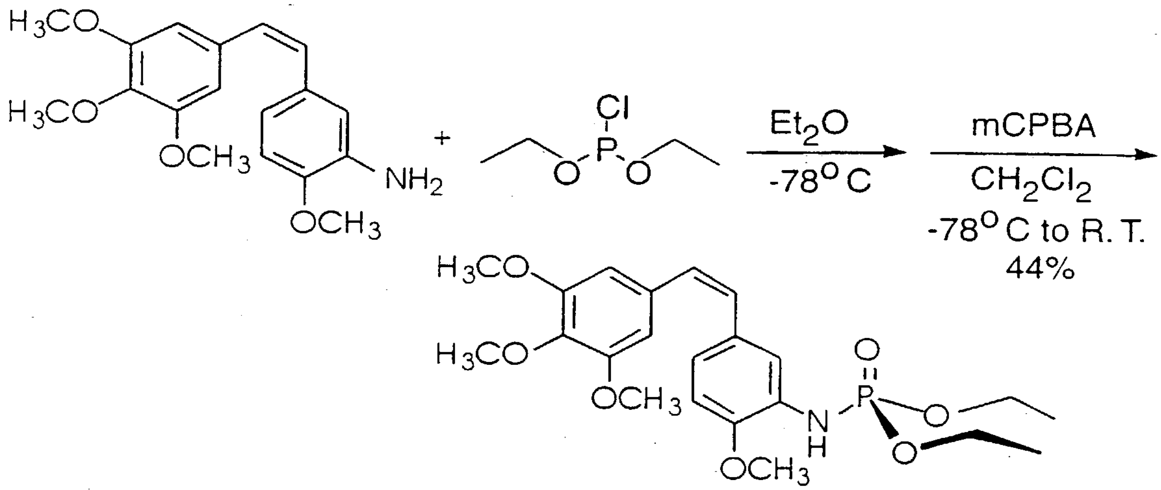


FIG. 19

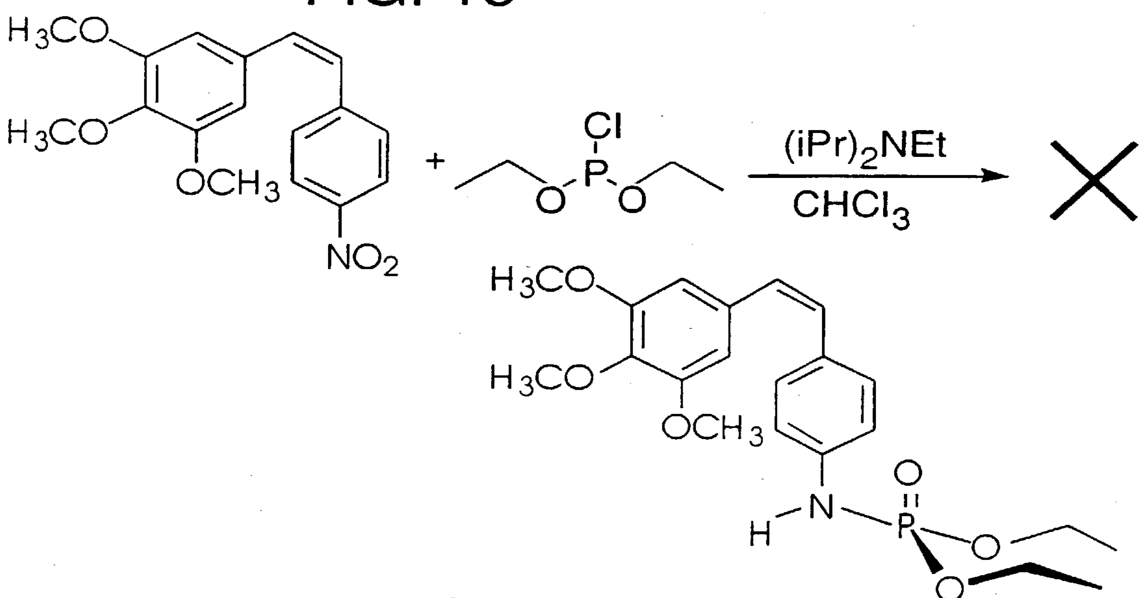


FIG. 20

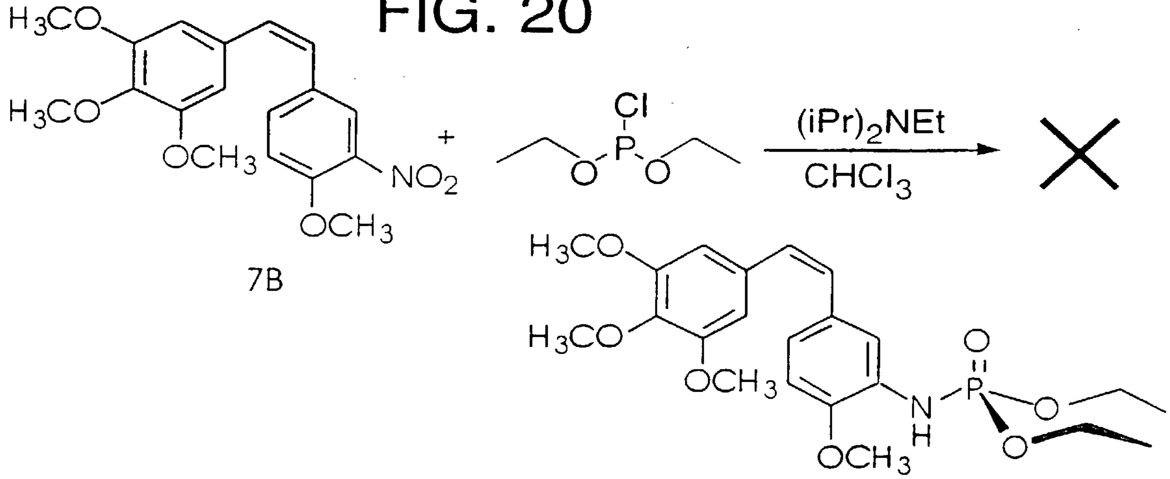


FIG. 21

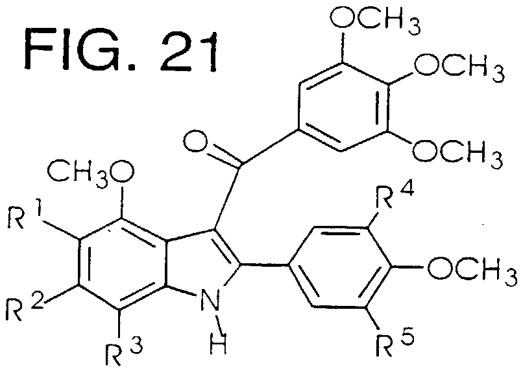


FIG. 22

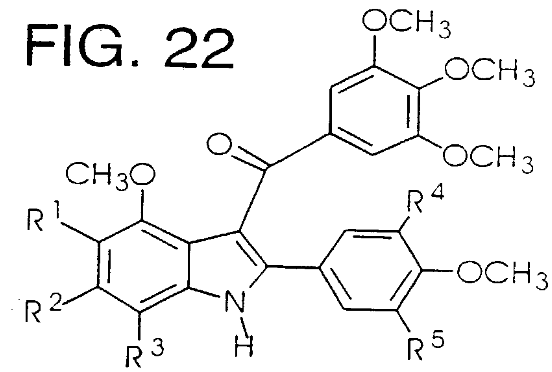


FIG. 23

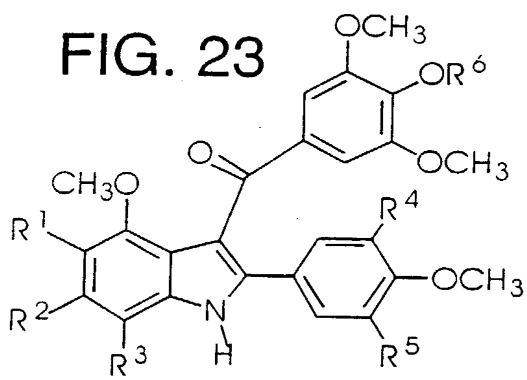


FIG. 24

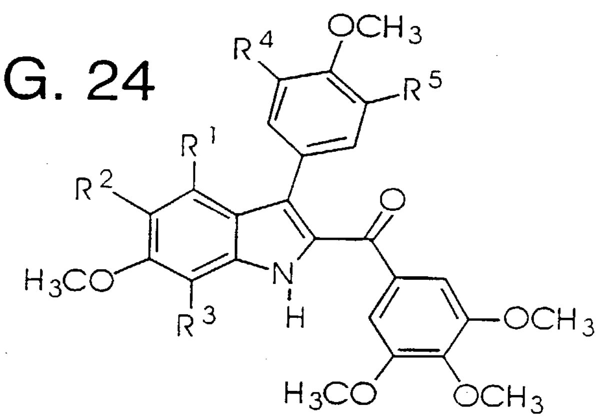


FIG. 25

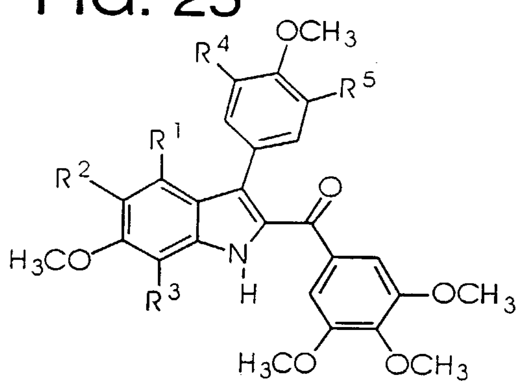


FIG. 26

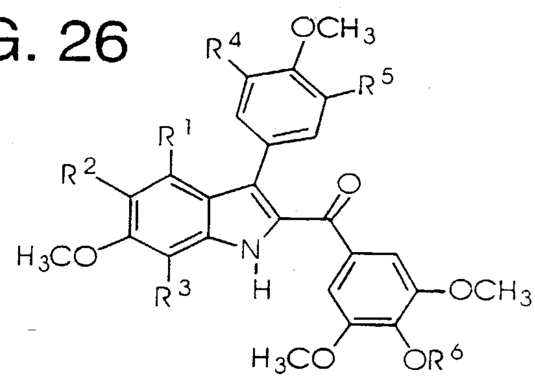


FIG. 27

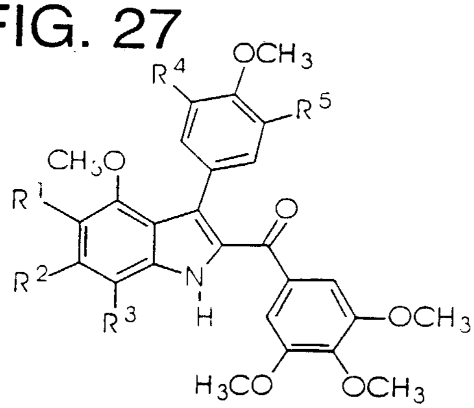


FIG. 28

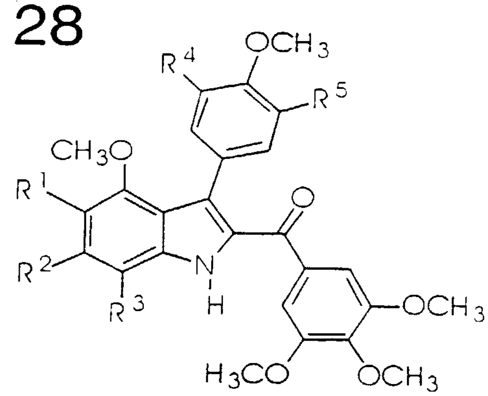


FIG. 29

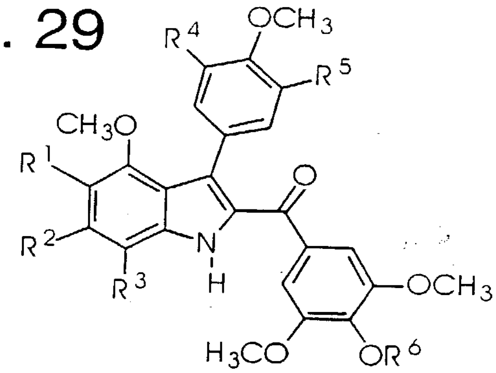


FIG. 30

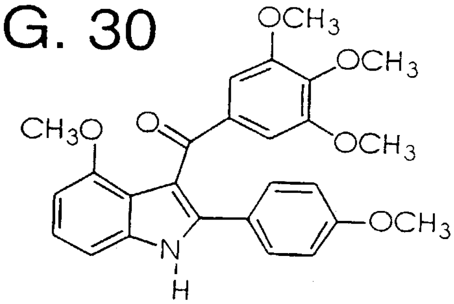


FIG. 31

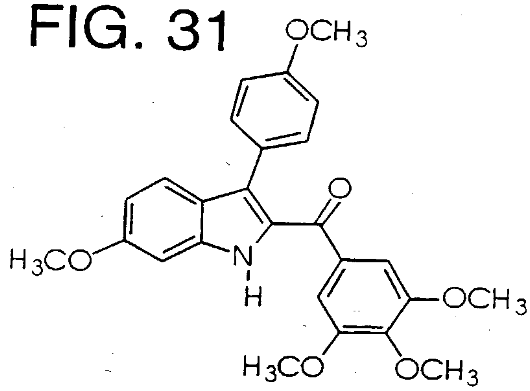


FIG. 32

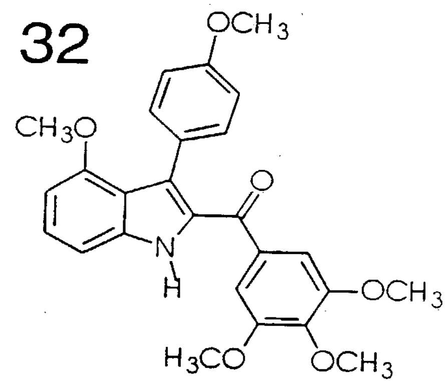


FIG. 33

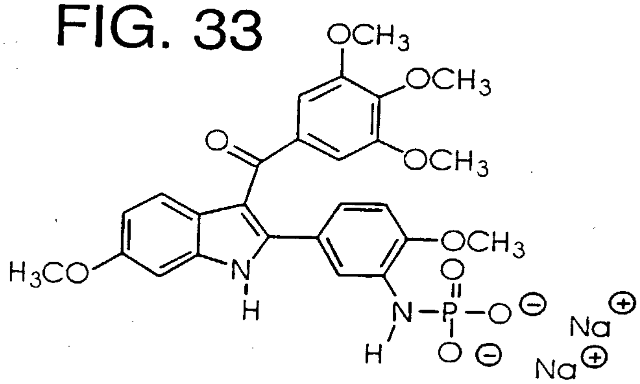


FIG. 34

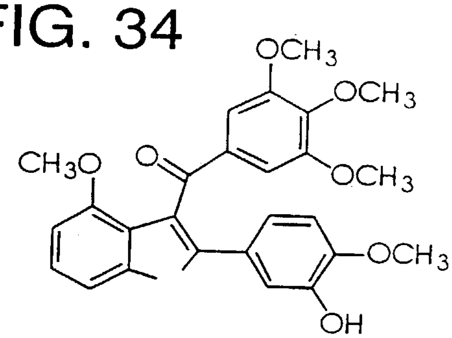


FIG. 35

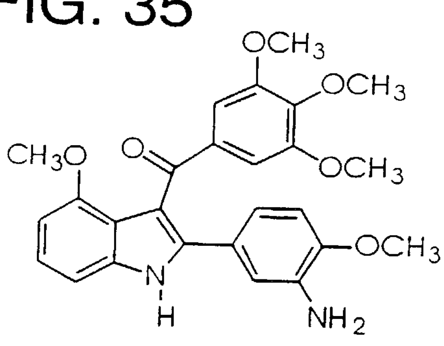


FIG. 36

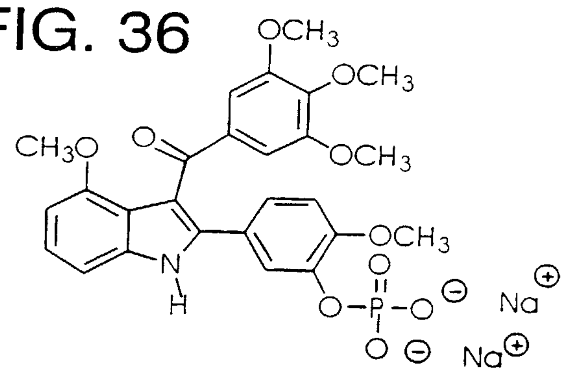


FIG. 37

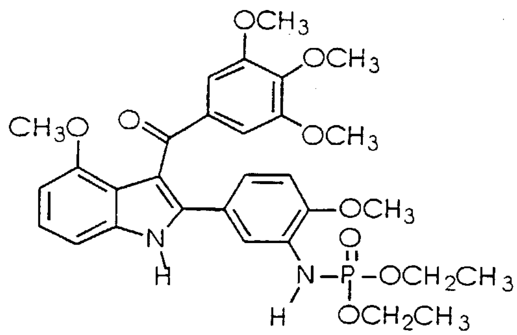


FIG. 38

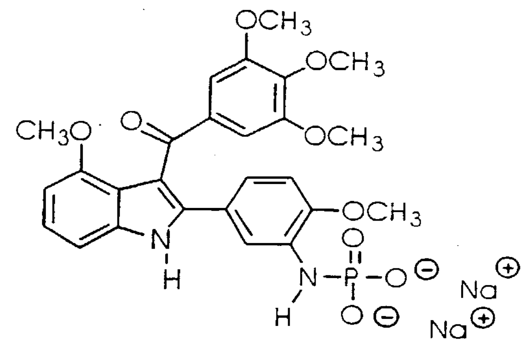


FIG. 39

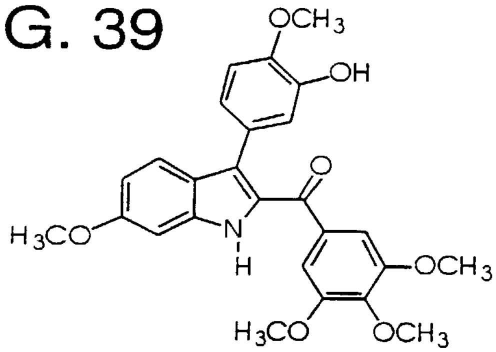


FIG. 40

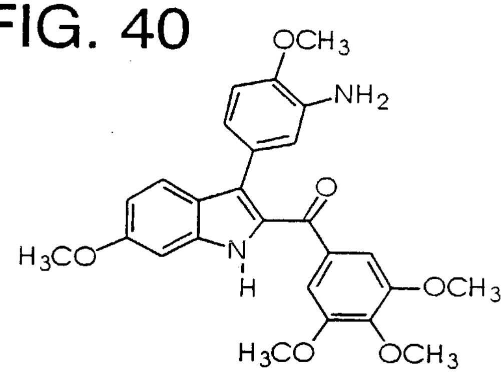


FIG. 41

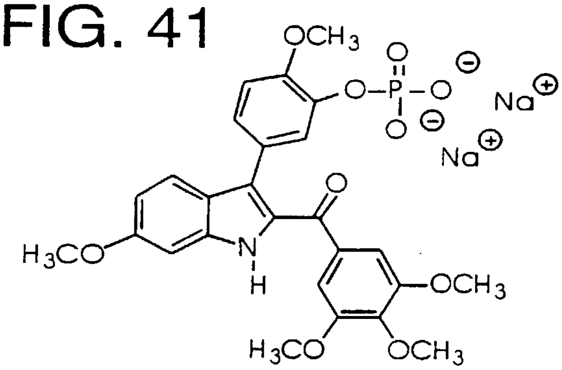


FIG. 42

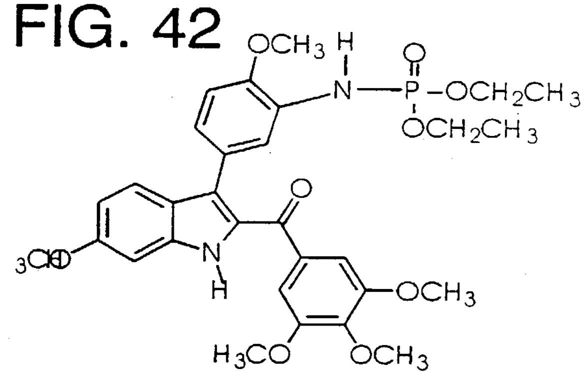


FIG. 43

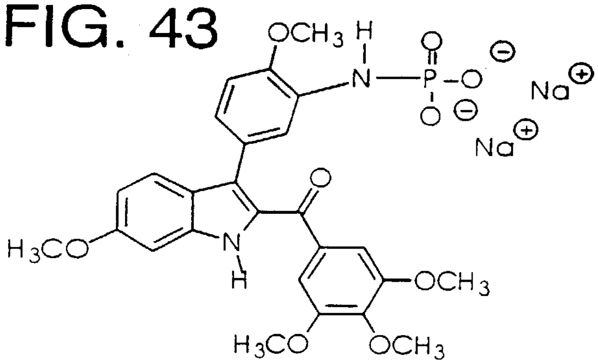


FIG. 44

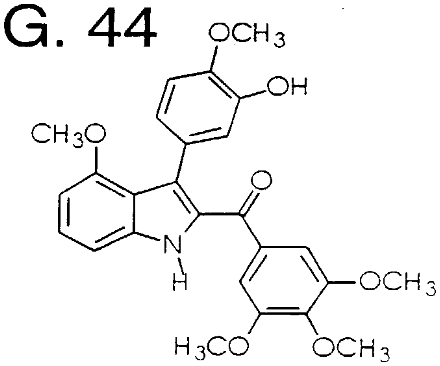


FIG. 45

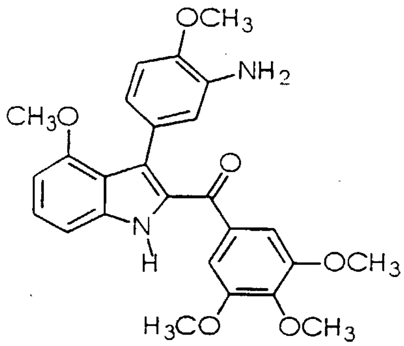


FIG. 46

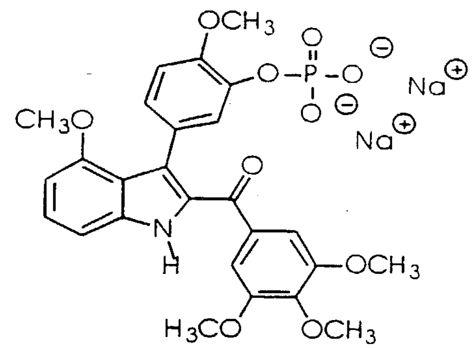


FIG. 47

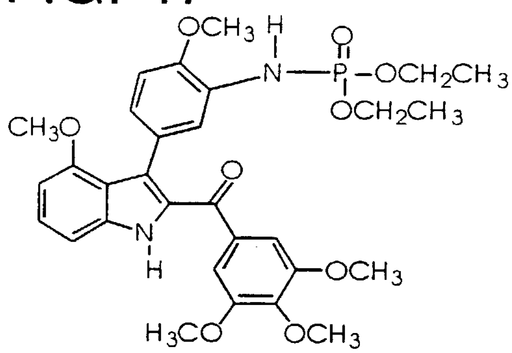


FIG. 48

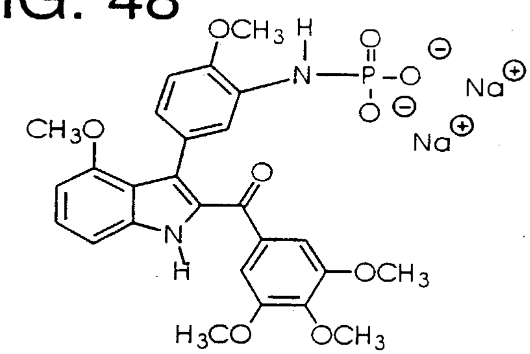


FIG. 49

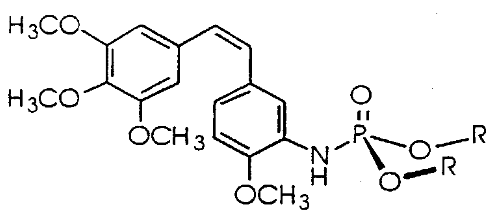


FIG. 50

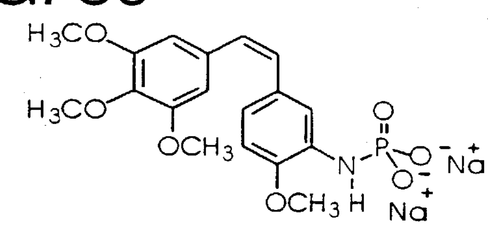
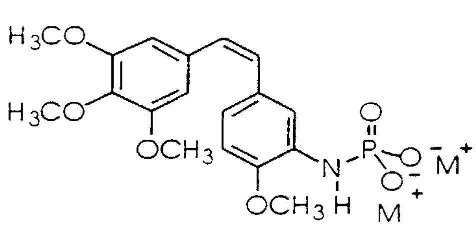


FIG. 51



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Kevin G. Pinney, et al SERIAL NO.: 10/070,484 FILING DATE: 06 MAR 2002 TITLE: **INDOLE-CONTAINING AND COMBRETASTATIN-RELATED ANTI-MITOTIC AND TUBULIN POLYMERIZATION AGENTS**	ATTY DKT NO. P-208614.9(PCT)(US)

COMBINED DECLARATION AND POWER OF ATTORNEY
(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION OR CIP)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type: (check one applicable item below)

[ ] original
[ ] design
[ ] supplemental

NOTE: If the declaration is for an International Application being filed as a divisional, continuation, or continuation-in-part application, do not check next item; check appropriate one of last three items.

[X] national stage of PCT

NOTE: If one of the following three items apply then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION or CIP.

[ ] divisional
[ ] continuation
[ ] continuation-in-part

INVENTORSHIP IDENTIFICATION

WARNING: If the inventors are each not the inventors of all the claims an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.

My residence, post office address and citizenship are as stated below next to my name, I believe I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITLE OF INVENTION: INDOLE-CONTAINING AND COMBRETASTATIN-RELATED ANTI-MITOTIC AND TUBULIN POLYMERIZATION AGENTS

SPECIFICATION IDENTIFICATION

the specification of which: *(complete (a), (b) or (c))*

(a) [X] is attached hereto
(b) [ ] was filed on ______________ as Serial No. ________________ or
[ ] Express Mail No. _____, as Serial No. not yet known, and was amended on _____ (if applicable).

NOTE: Amendments filed after the original papers are deposited with the PTO which contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 CFR 1.67.

(c) [X] was described and claimed in PCT International Application No. PCT/US00/25408 filed on September 15, 2000.

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information:

[X] which is material to the patentability as defined in 37, Code of Federal Regulations, § 1.56.

(also check the following, if desired)

[ ] and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable examiner would consider it important in deciding whether to allow the application to issue as a patent, and

[ ] In compliance with this duty there is attached an information disclosure statement in accordance with 37 CFR 1.98.

PRIORITY CLAIM (35 U.S.C. § 119)

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) [ ] no such applications have been filed.
(e) [X] such applications have been filed as follows:

NOTE: Where item (c) is entered above and the International Application which designated the U.S. claimed priority, check item (e), enter the details below and make the priority claim.

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119

COUNTRY	APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)	PRIORITY CLAIMED UNDER 37 USC 119
USA	PCT/US00/25408	15 SEP 00	[X] YES [ ] NO

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

COUNTRY	APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)	PRIORITY CLAIMED UNDER 37 USC 119
			[ ] YES [ ] NO

NOTE: If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete "Added Pages to Combined Declaration and Power of Attorney for Divisional, Continuation, or C-I-P Application" for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.

POWER OF ATTORNEY

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(List name and registration number.)*

Daniel D. Chapman	Registration No. 32,726
William B. Nash	Registration No. 33,743
Mark H. Miller	Registration No. 29,197
7 Thomas Sisson	Registration No. 29,348
Cline H. White	Registration No. 45,213
Richard R. Ruble	Registration No. 45,720
Daniel D. Hodgins	Registration No. 31,026

(check the following item, if applicable)

[ ] Attached as part of this declaration and power of attorney is the authorization of the above-named attorney(s) to accept and follow instructions from my representative(s).

SEND CORRESPONDENCE TO:

Daniel S. Hodgins
JACKSON WALKER, LLP
112 E. Pecan, Suite 2100
an Antonio, Texas 78205

DIRECT TELEPHONE CALLS TO:

Name and Telephone Number)
Daniel S. Hodgins
(210) 978-7759

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURES

1-00

FULL NAME OF SOLE/FIRST INVENTOR	Kevin G. Pinney
INVENTOR'S SIGNATURE	K. G. Pinney
DATE OF EXECUTION	9-17-02
COUNTRY OF CITIZENSHIP	U.S.
RESIDENTIAL ADDRESS	~~100 Russell Lane~~ ~~Hewitt, TX 76643~~ 717 Topeka, Woodway, TX 76712

FULL NAME OF SECOND INVENTOR	Feng Wang
INVENTOR'S SIGNATURE	
DATE OF EXECUTION	
COUNTRY OF CITIZENSHIP	U.S.
RESIDENTIAL ADDRESS	600 American Avenue, Apt. C304 King of Prussia, PA 19406

FULL NAME OF SECOND INVENTOR	Maria Del Pilar Mejia
INVENTOR'S SIGNATURE	
DATE OF EXECUTION	
COUNTRY OF CITIZENSHIP	U.S.
RESIDENTIAL ADDRESS	9999 Linda Lane, Apt. GE Des Plaines, IL 60016

CHECK PROPER BOX(ES) IF ANY OF THE FOLLOWING ADDED PAGE(S) FORM A PART OF THIS DECLARATION

[ ] Signature for subsequent joint inventors. Number of pages added: ____.

[ ] Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added: ____.

(3)

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURES

FULL NAME OF SOLE/FIRST INVENTOR	Kevin G. Pinney
INVENTOR'S SIGNATURE	
DATE OF EXECUTION	
COUNTRY OF CITIZENSHIP	U.S.
RESIDENTIAL ADDRESS	100 Russell Lane Hewitt, TX 76643

2-00

FULL NAME OF SECOND INVENTOR	Feng Wang
INVENTOR'S SIGNATURE	Feng Wang
DATE OF EXECUTION	09/20/02
COUNTRY OF CITIZENSHIP	U.S.
RESIDENTIAL ADDRESS	600 American Avenue, Apt. C304 King of Prussia, PA 19406

FULL NAME OF SECOND INVENTOR	Maria Del Pilar Mejia
INVENTOR'S SIGNATURE	
DATE OF EXECUTION	
COUNTRY OF CITIZENSHIP	U.S.
RESIDENTIAL ADDRESS	9999 Linda Lane, Apt. GE Des Plaines, IL 60016

CHECK PROPER BOX(ES) IF ANY OF THE FOLLOWING ADDED PAGE(S) FORM A PART OF THIS DECLARATION

[ ] Signature for subsequent joint inventors. Number of pages added: ______.

[ ] Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added: ______.

Over

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURES

FULL NAME OF SOLE/FIRST INVENTOR	Kevin G. Pinney
INVENTOR'S SIGNATURE	
DATE OF EXECUTION	
COUNTRY OF CITIZENSHIP	U.S.
RESIDENTIAL ADDRESS	100 Russell Lane Hewitt, TX 76643

FULL NAME OF SECOND INVENTOR	Feng Wang
INVENTOR'S SIGNATURE	
DATE OF EXECUTION	
COUNTRY OF CITIZENSHIP	U.S.
RESIDENTIAL ADDRESS	600 American Avenue, Apt. C304 King of Prussia, PA 19406

FULL NAME OF SECOND INVENTOR	Maria Del Pilar Mejia
INVENTOR'S SIGNATURE	[signature]
DATE OF EXECUTION	09-20-02
COUNTRY OF CITIZENSHIP	~~U.S.~~ Colombia
RESIDENTIAL ADDRESS	~~9999 Linda Lane, Apt. 6E~~ 1106 Bull Street ~~Des Plaines, IL 60016~~ Normal, IL 61761

CHECK PROPER BOX(ES) IF ANY OF THE FOLLOWING ADDED PAGE(S) FORM A PART OF THIS DECLARATION

[ ] Signature for subsequent joint inventors. Number of pages added: _____.

[ ] Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added: _____.

over

[ ] Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.45. Number of pages added: _____.

[ ] Added page for signature by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 CFR 1.47).

[X] Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (CIP) application. Number of pages added: two (2).

[ ] Authorization of attorney(s) to accept and follow instructions from representative.

If no further pages form a part of this Declaration then end this Declaration with this page and check the following item:

[X] This declaration ends with this page.

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited on the date shown below with the United States Postal Service in an envelope addressed to the "Commissioner of Patents and Trademarks, Washington, D.C. 20231", as follows:

37 CFR 1.8(a)	37 CFR 1.10
[ ] With sufficient postage as First Class Mail.	[X] As "Express Mail Post Office to Addressee", Mailing Label No. EL661644269US.
Date: ________, 20__	Date: 10-11-, 2002

ElvA J. Abundis
Printed Name of Person Mailing Paper or Fee

[signature]
Signature of Person Mailing Paper or Fee

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CIP APPLICATION

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S) UNDER 35 U.S.C. §120

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 USC §120					
U.S. APPLICATIONS			Status (Check one)		
U.S. APPLICATIONS		U.S. FILING DATE	Patented	Pending	Abandoned
1. 60/154,639		09/17/99		X	
2.					
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLI-CATION NO.	PCT FILING DATE	U.S. SERIAL NOS. ASSIGNED (if any)			
3.PCT/US00/25408	09/15/00			X	
4.					

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